Desmond 2.2 Tutorial Workshop

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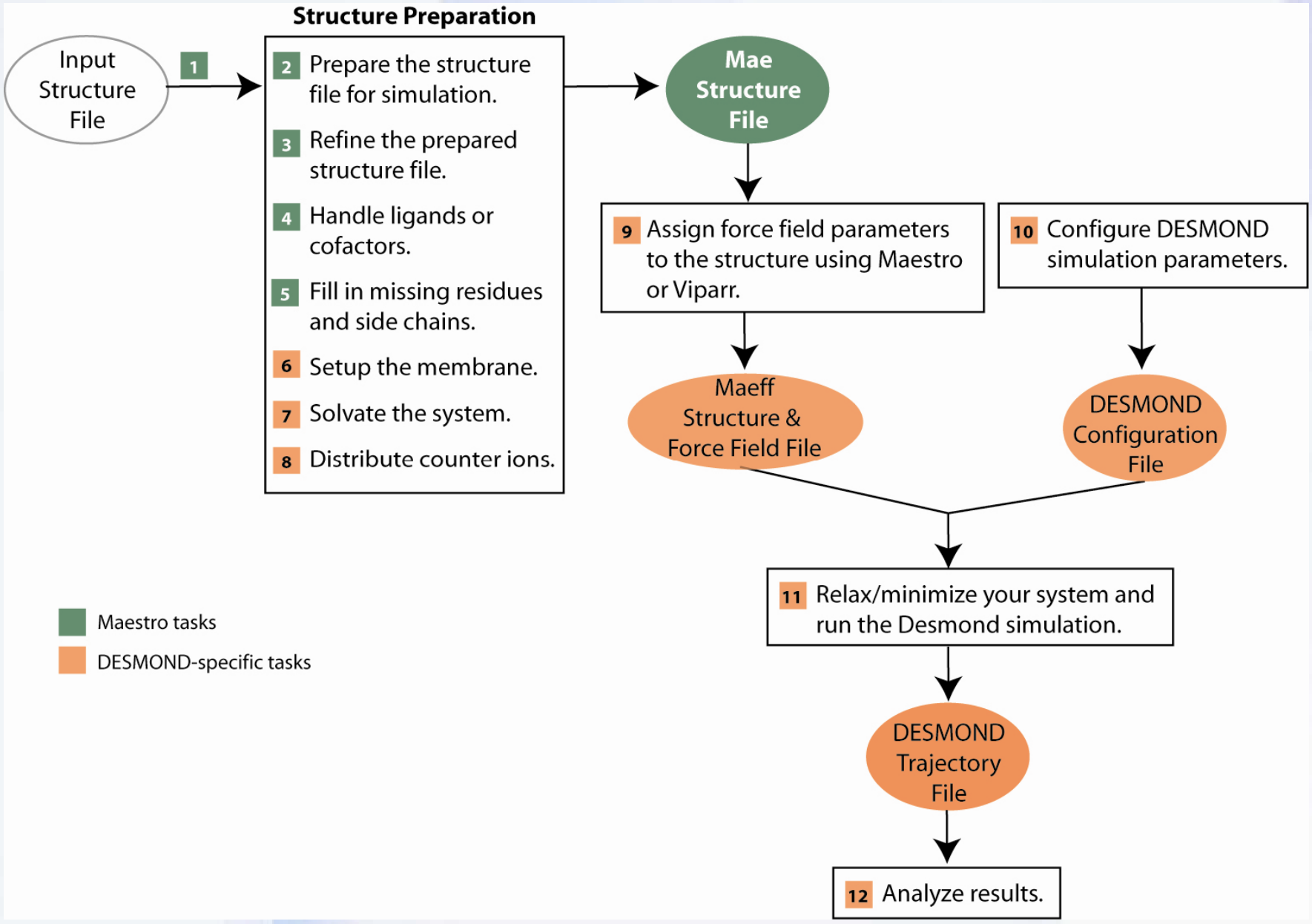
Introducing Desmond

- Molecular dynamics simulation system
- Integrated with Maestro modeling environment, compatible with VMD visualization & analysis tools
- Long-scale simulations of small molecules, proteins, nucleic acids, and membrane systems
- Study interactions of complex molecular assemblies
- Advanced simulation techniques: FEP, simulated annealing, replica exchange, steered dynamics

Simulation Steps

1. Import a molecular structure file
2. Prepare/refine the structure for simulation
3. (Immerse the protein in the membrane)
4. Generate a solvated system with PBC
5. Neutralize the system (and add salt)
6. Assign force field parameters
7. Configure simulation parameters
8. Launch Desmond job

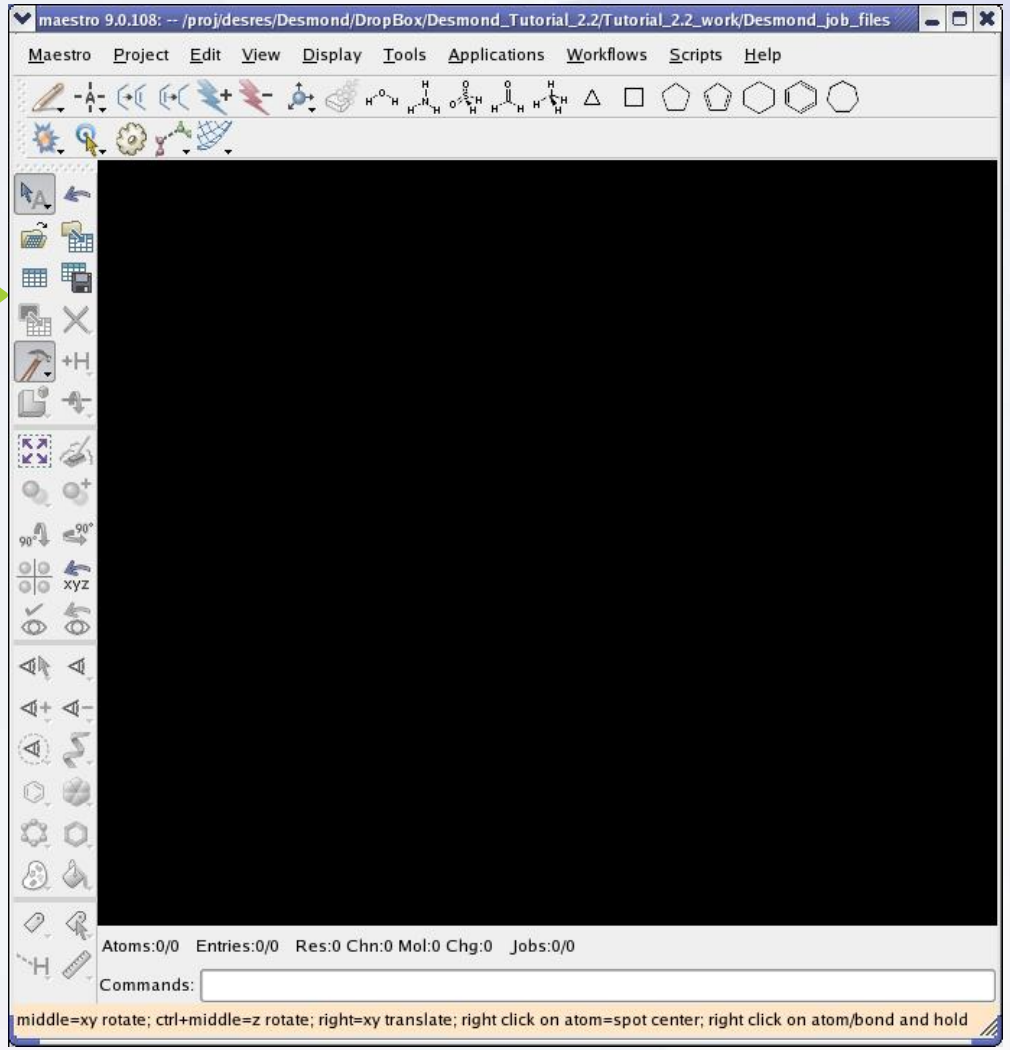

Structure Preparation
Input Structure File
1
2 Prepare the structure file for simulation.
3 Refine the prepared structure file.
4 Handle ligands or cofactors.
5 Fill in missing residues and side chains.
6 Setup the membrane.
7 Solvate the system.
8 Distribute counter ions.
Mae Structure File
9 Assign force field parameters to the structure using Maestro or Viparr.
10 Configure DESMOND simulation parameters.
Maeff Structure & Force Field File
DESMOND Configuration File
11 Relax/minimize your system and run the Desmond simulation.
DESMOND Trajectory File
12 Analyze results.
Maestro tasks
DESMOND-specific tasks

Start Maestro

- $SCHRODINGER/maestro
- $SCHRODINGER/maestro –SGL (over network)

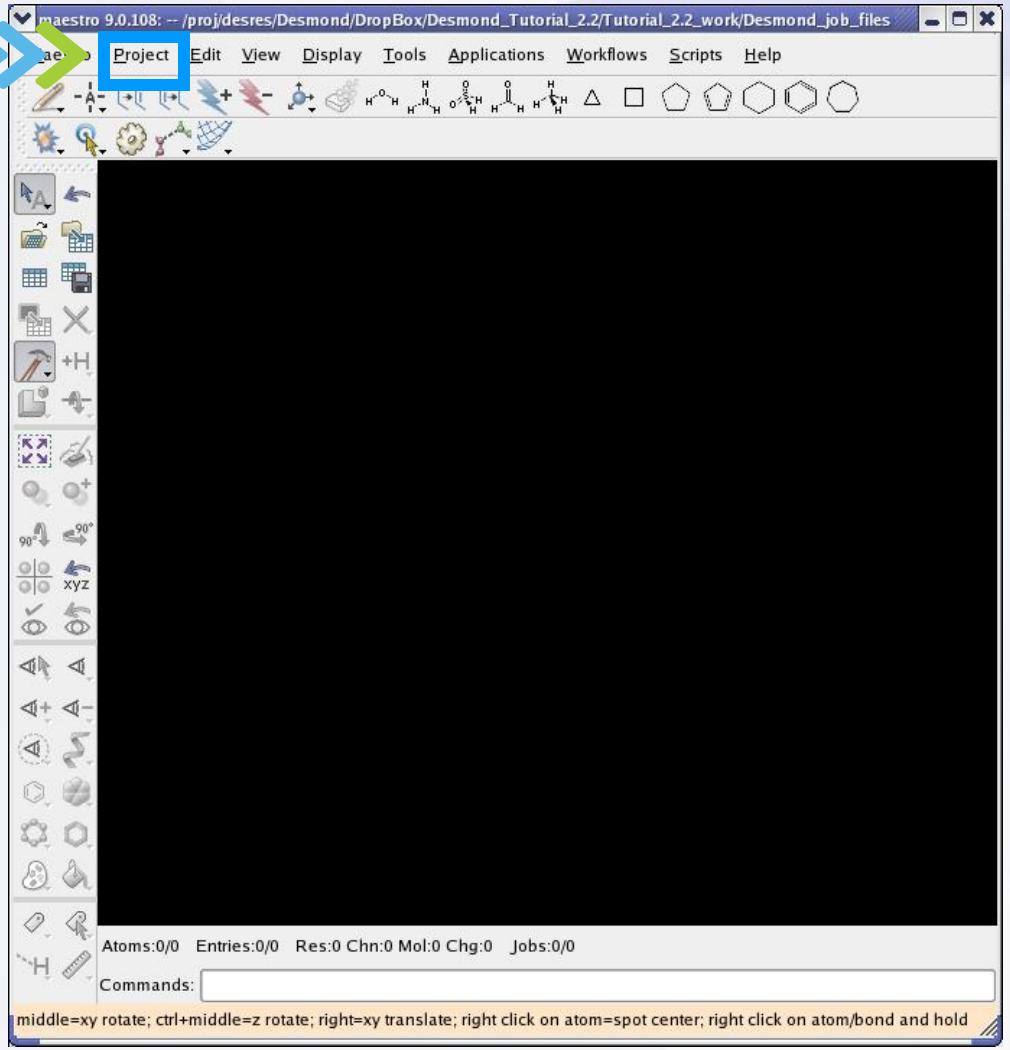
Start Maestro

Maestro main window >>>>>


maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:0/0 Entries:0/0 Res:0 Chn:0 Mol:0 Chg:0 Jobs:0/0
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold

Import a structure file

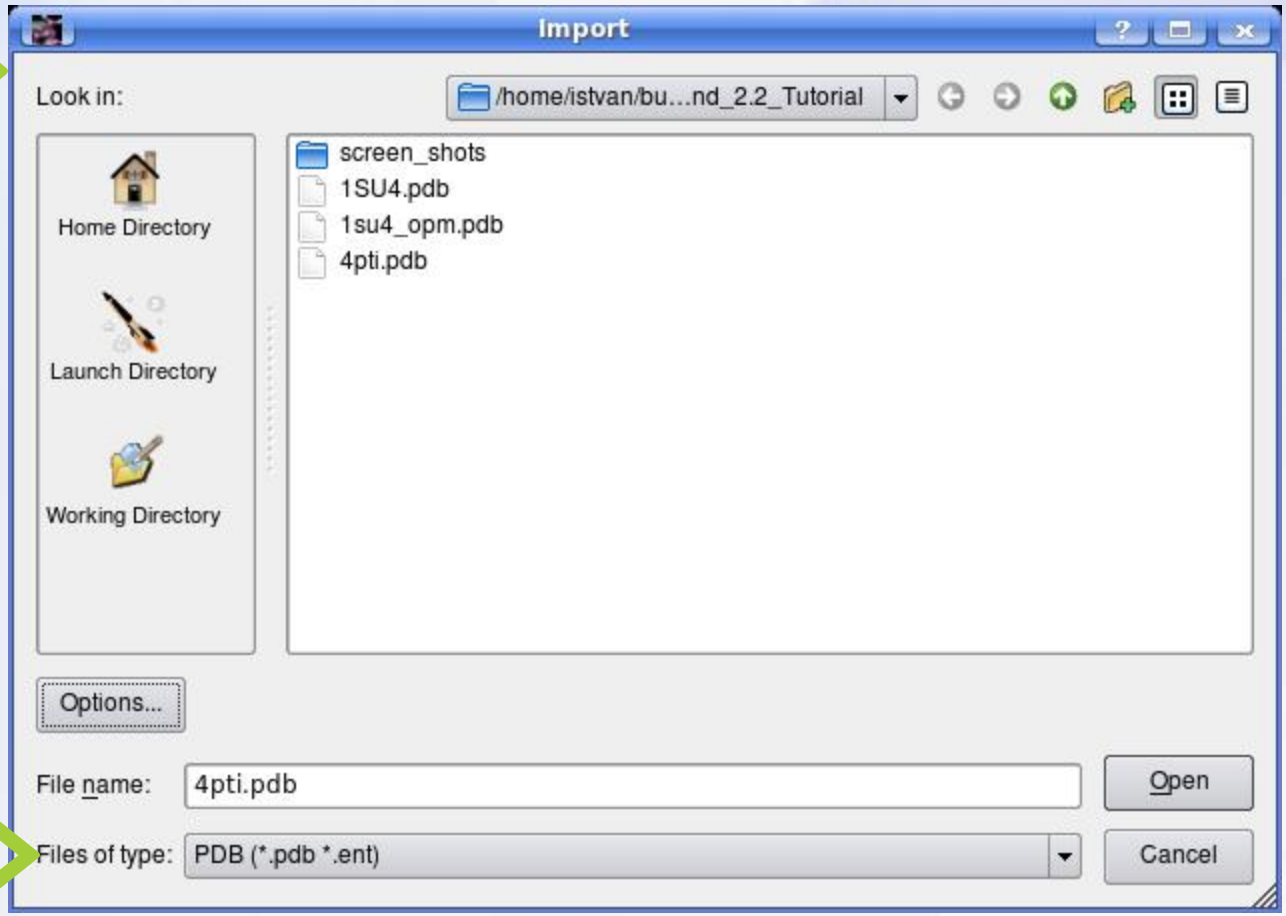
Select Project > Import Structures



Import a structure file

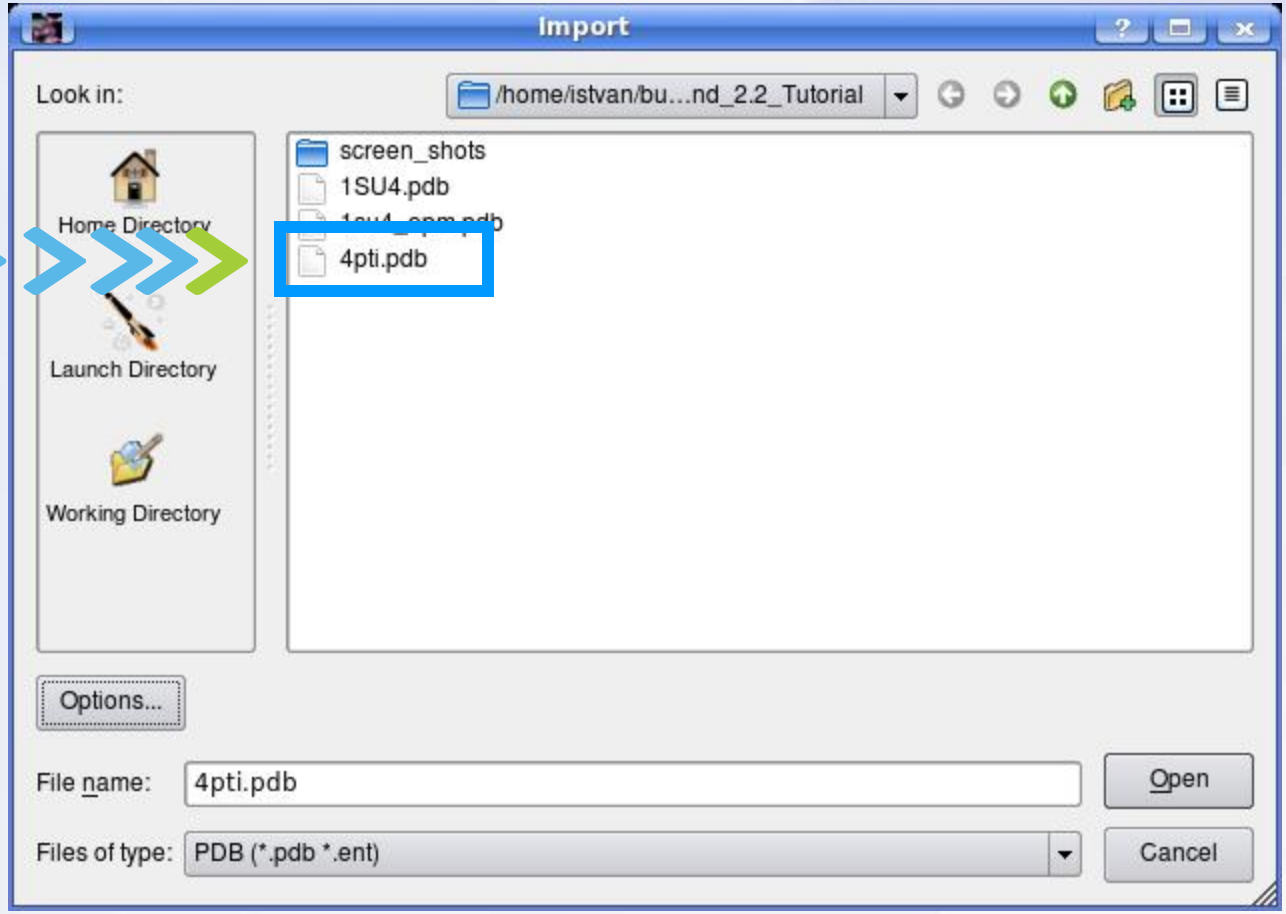
The Import panel appears

Select PDB



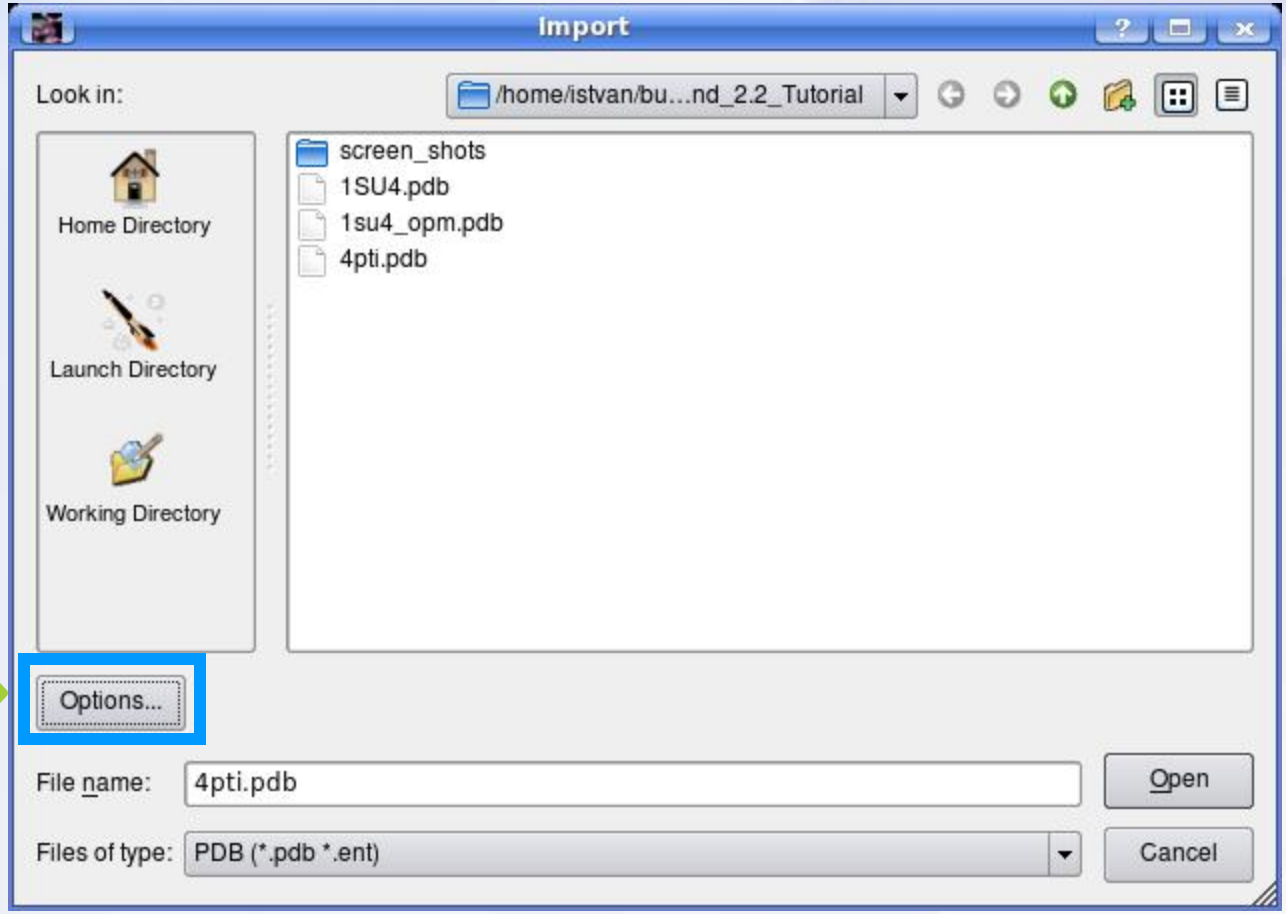
Import a structure file

Select the structure file you want to import



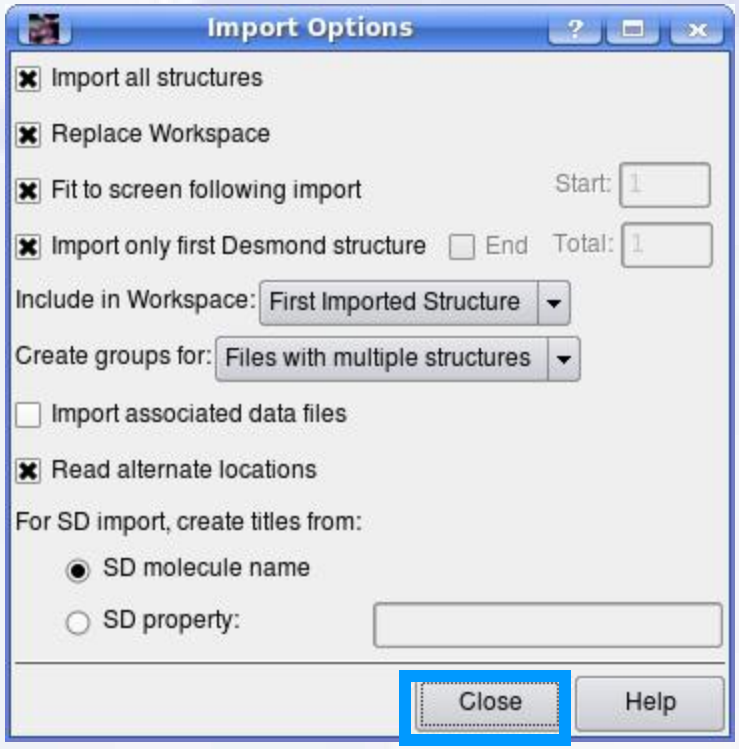
Import a structure file

Click Options



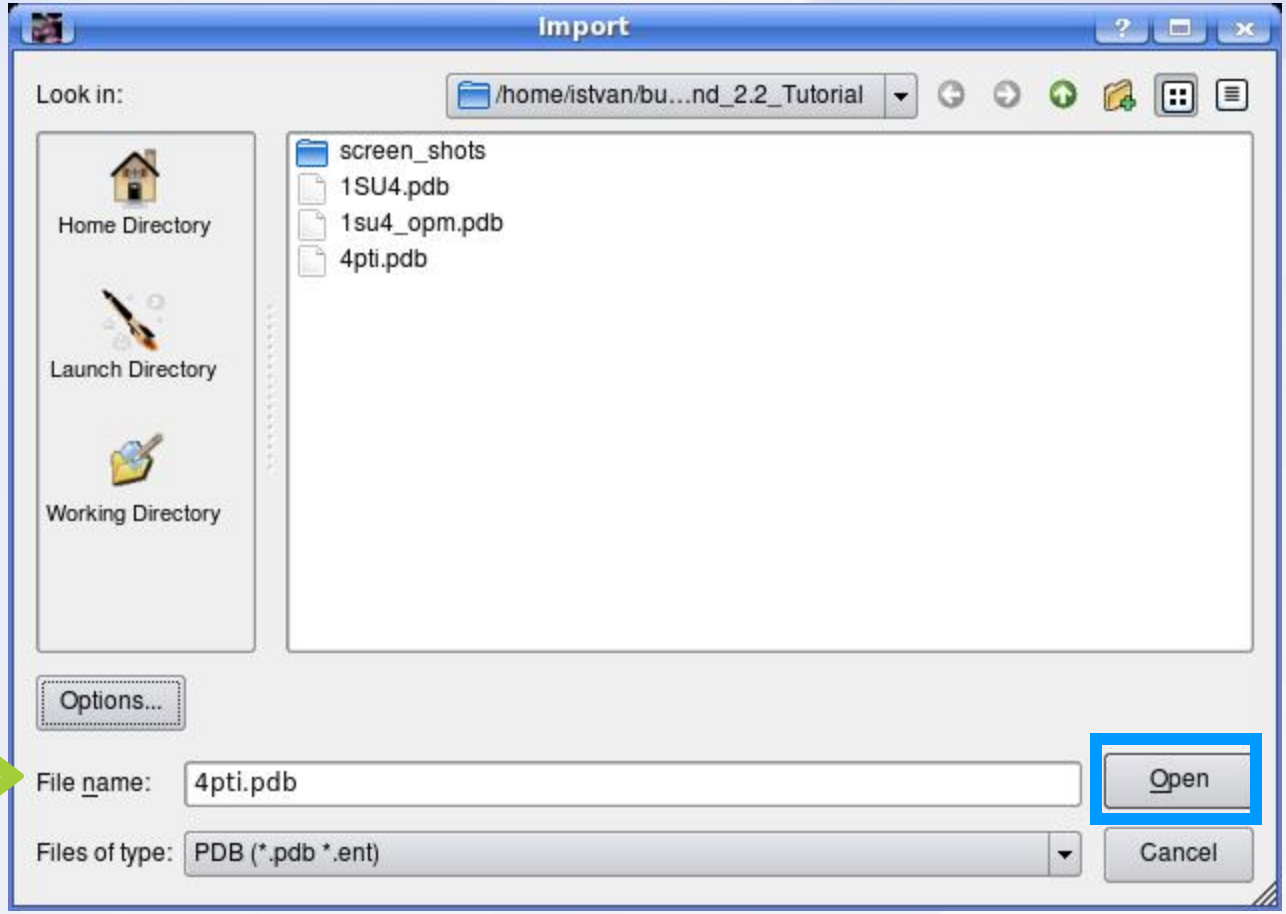
Import a structure file

The Import Options panel appears



Click Close when done

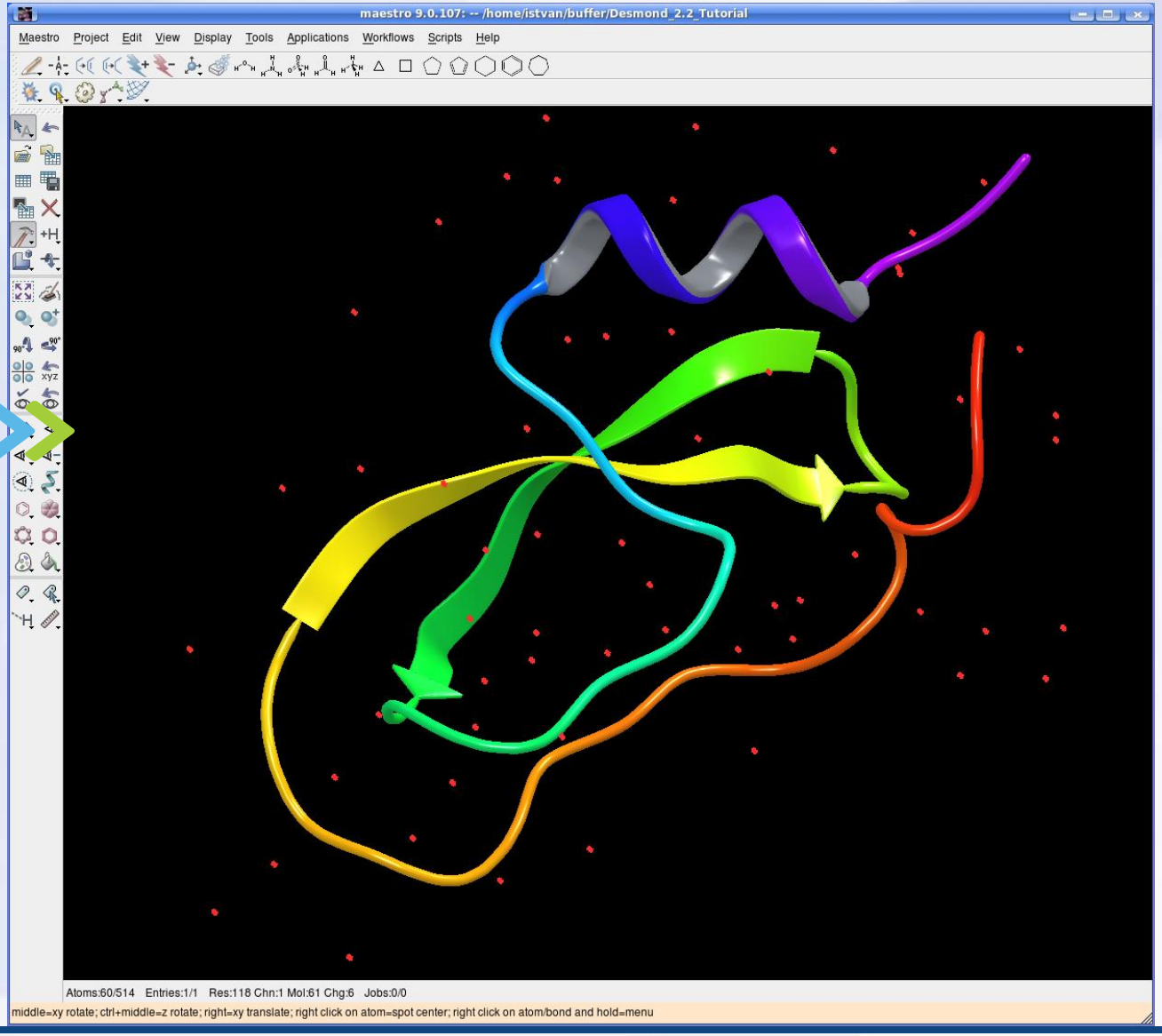
Import a structure file



Click Open >>>>

Import a structure file

The protein structure appears in Maestro


maestro 9.0.107: -- /home/istvan/buffer/Desmond_2.2_Tutorial
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:60/514 Entries:1/1 Res:118 Chn:1 Mol:61 Chg:6 Jobs:0/0
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

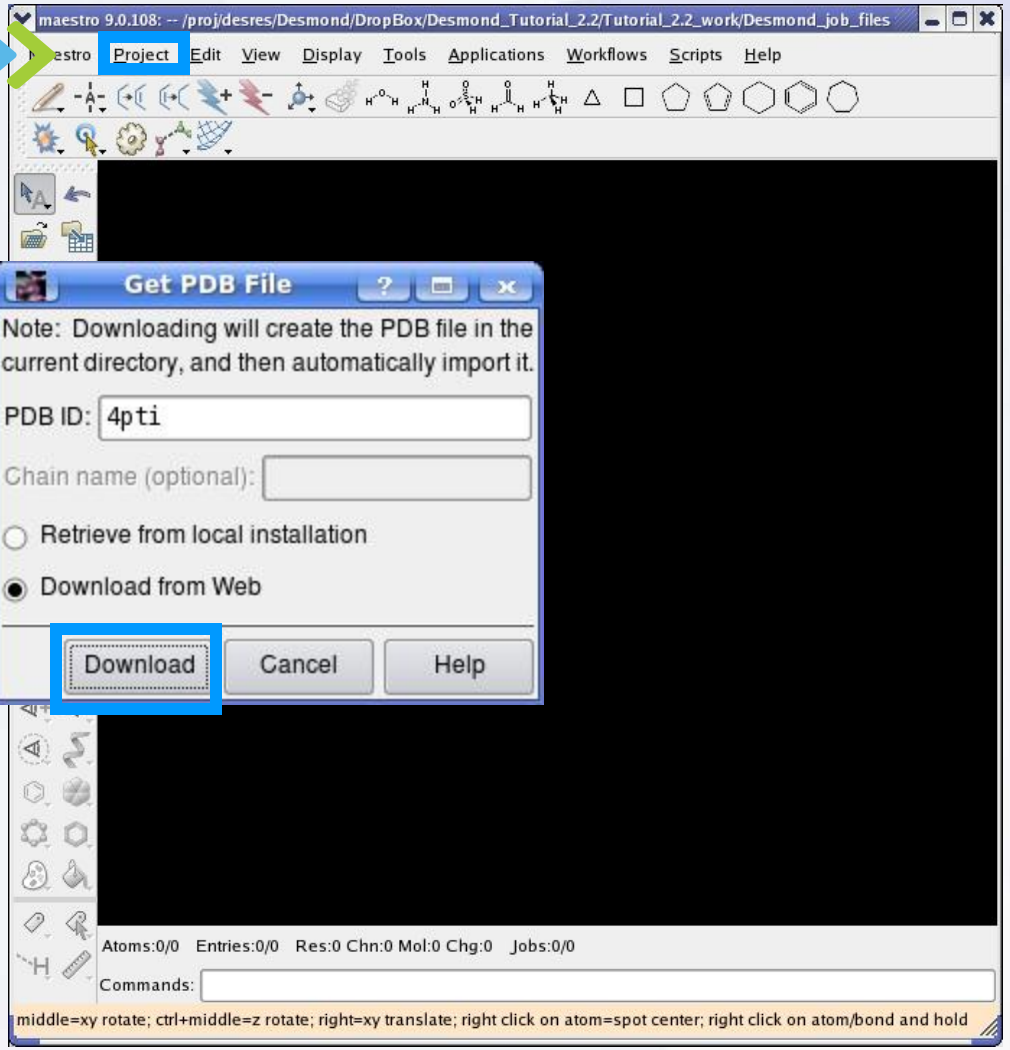
Download a structure file

Select Project > Get PDB

Enter identifier

Select the Download from Web option

Click Download



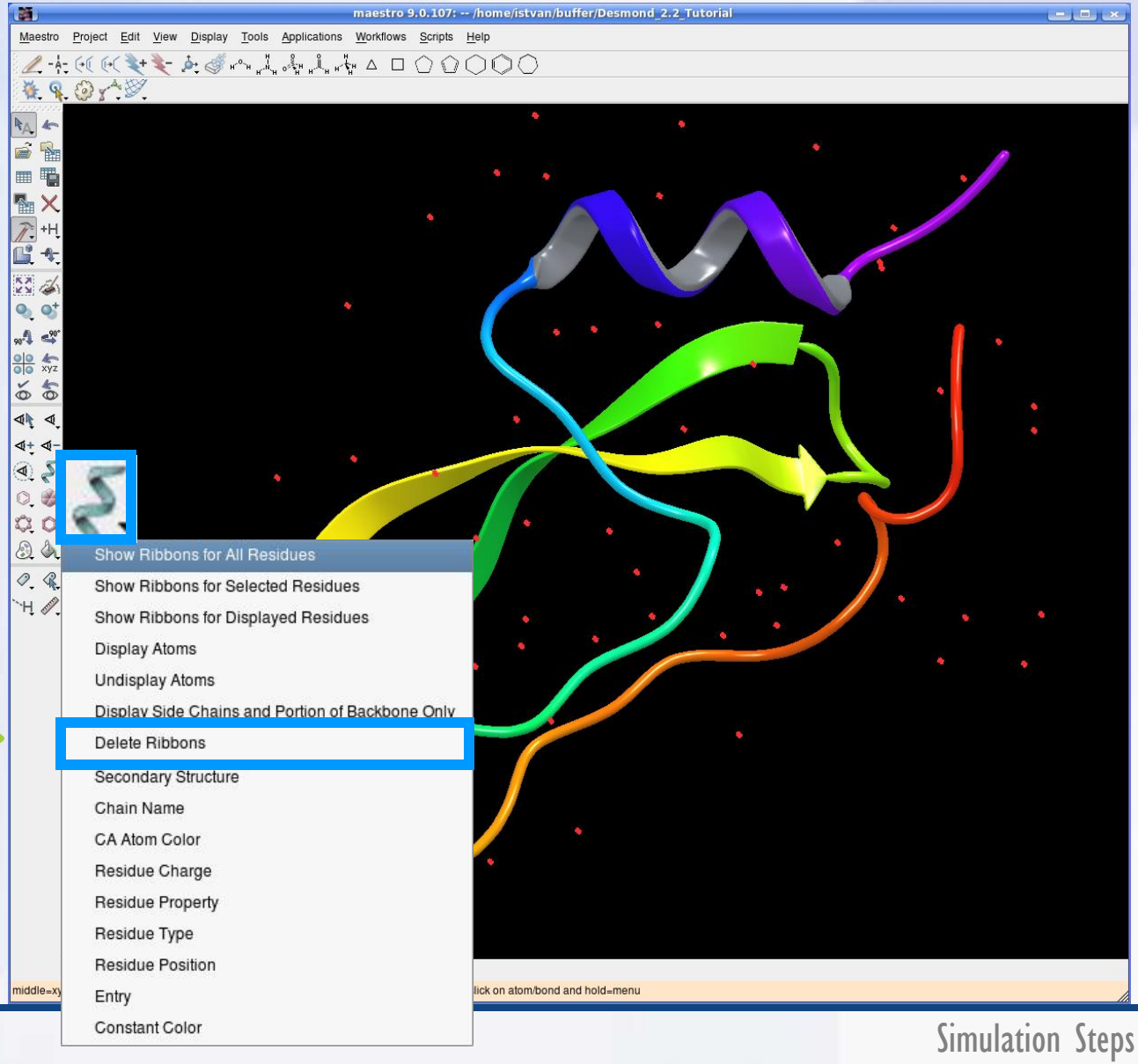
Prepare for simulation

- Delete ribbons
- Switch to ball-and-stick view for clarity
- Color carbons green in the structure
- Prepare the protein for simulation:
  - Assign bond orders
  - Add hydrogen atoms
  - Remove crystal waters
  - Etc.

Prepare for simulation: Delete ribbons

Click the Ribbon button…

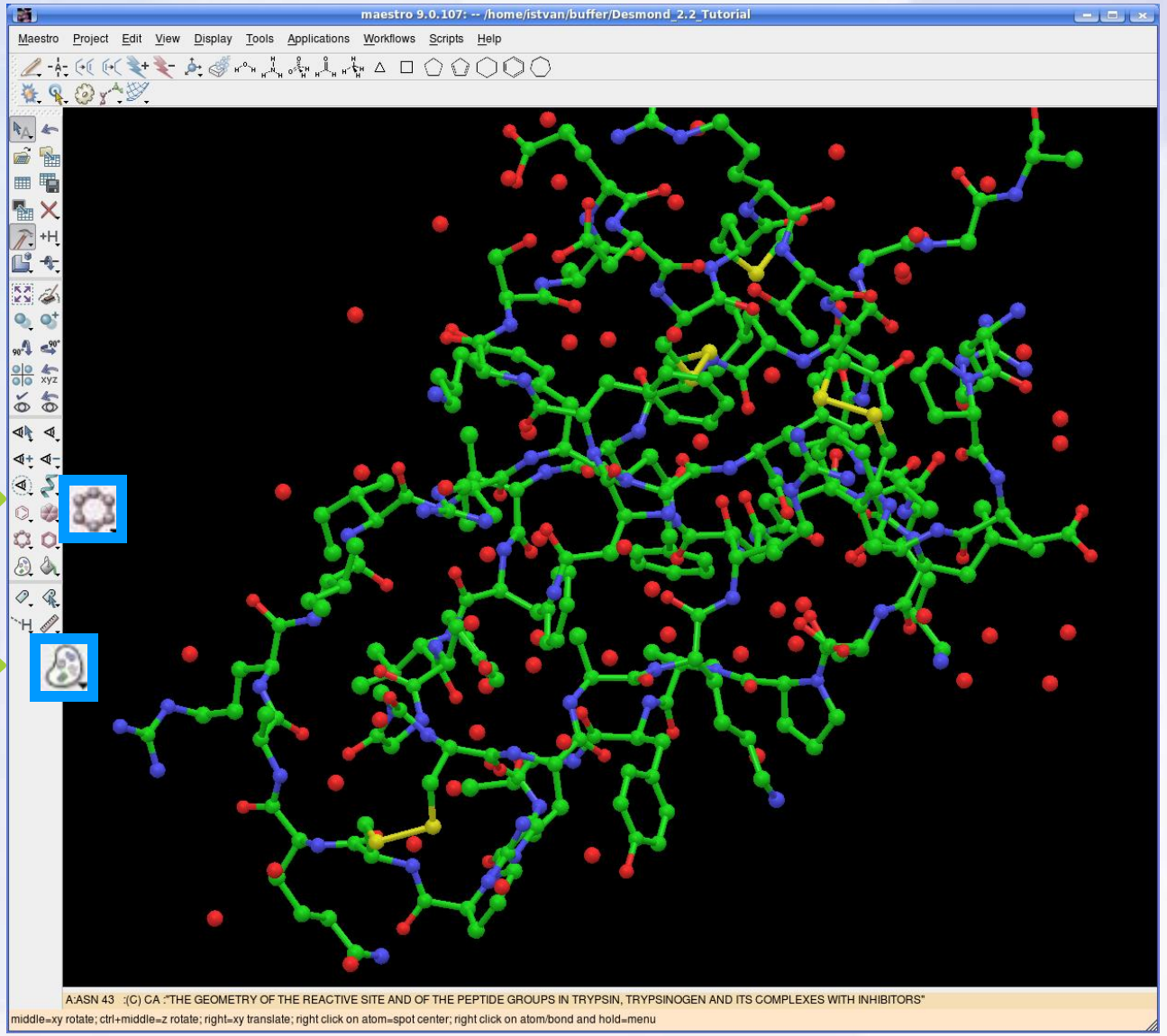
Select Delete Ribbons



Prepare for simulation: Change view

Switch to ball-and-stick view for clarity

Double-click
Ball & Stick
Click Color
By Elements
(green carbon)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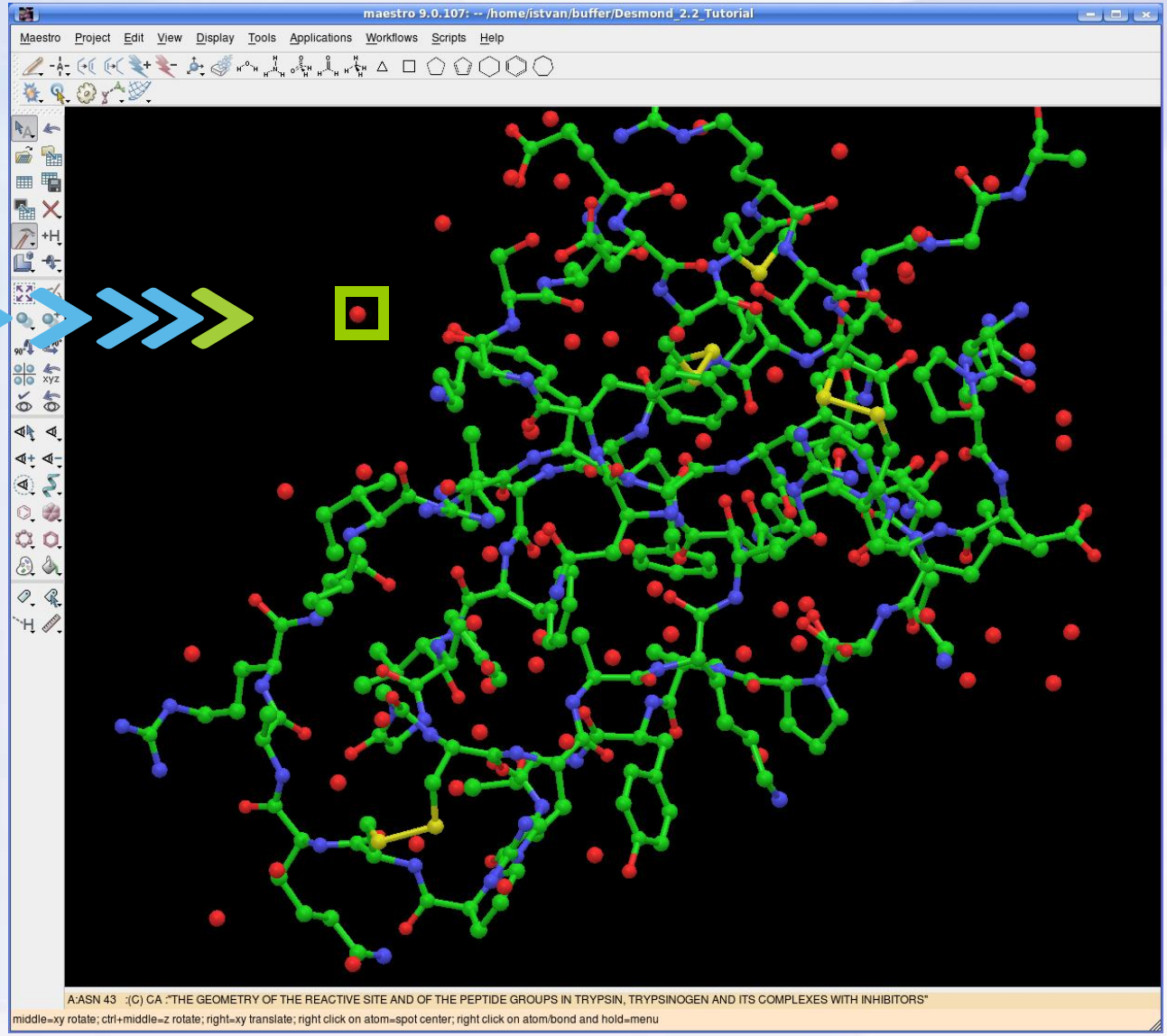
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
A:ASN 43 :(C) CA :"THE GEOMETRY OF THE REACTIVE SITE AND OF THE PEPTIDE GROUPS IN TRYPSIN, TRYPSINOGEN AND ITS COMPLEXES WITH INHIBITORS"
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Prepare for simulation: Change view

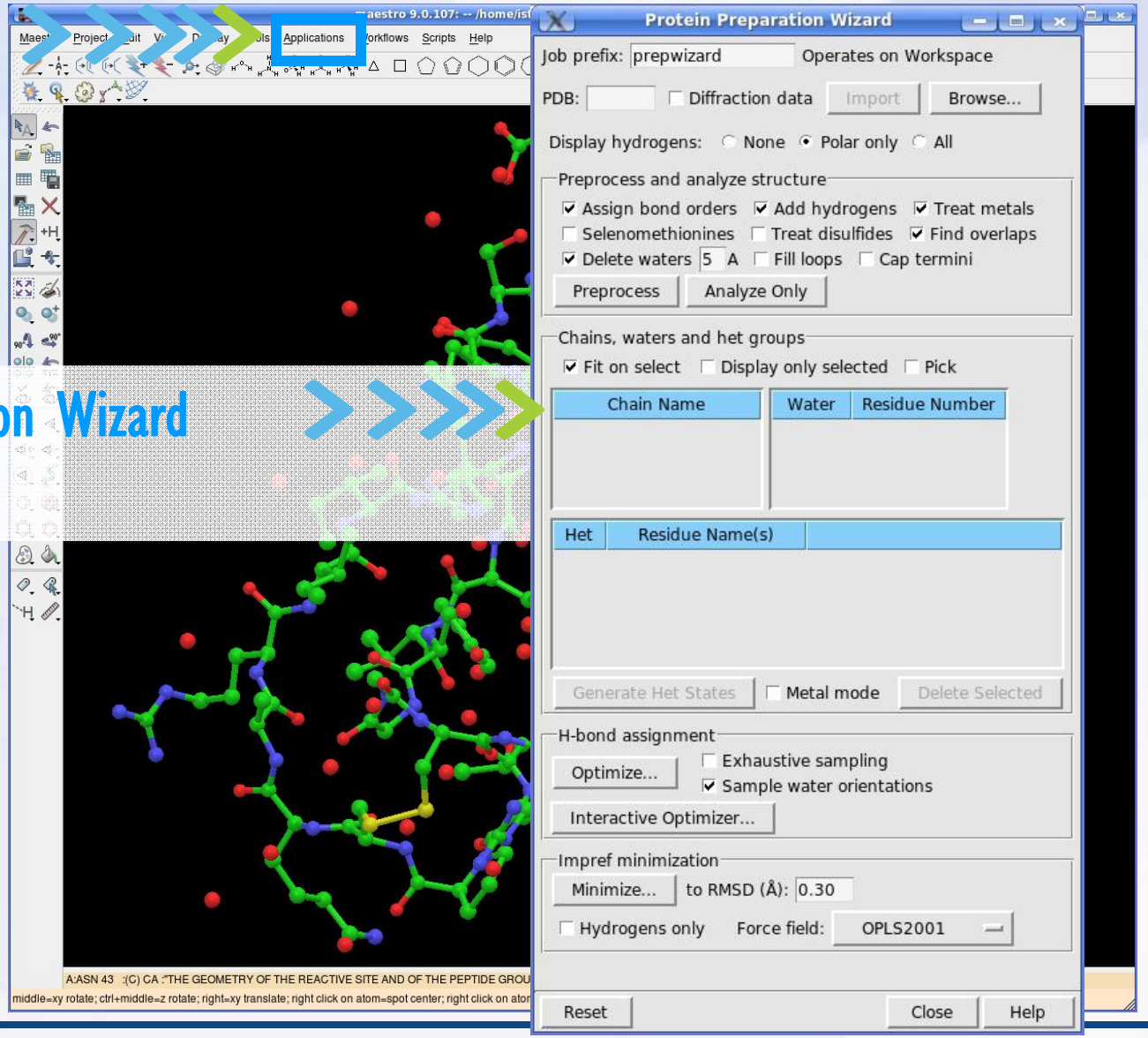
Red dots represent crystal water oxygen atoms


maestro 9.0.107: -- /home/istvan/buffer/Desmond_2.2_Tutorial
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
A:ASN 43 :(C) CA :"THE GEOMETRY OF THE REACTIVE SITE AND OF THE PEPTIDE GROUPS IN TRYPSIN, TRYPSINOGEN AND ITS COMPLEXES WITH INHIBITORS"
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Prepare for simulation: Prepare protein

Select Workflows > Protein Preparation Wizard

The Protein Preparation Wizard appears

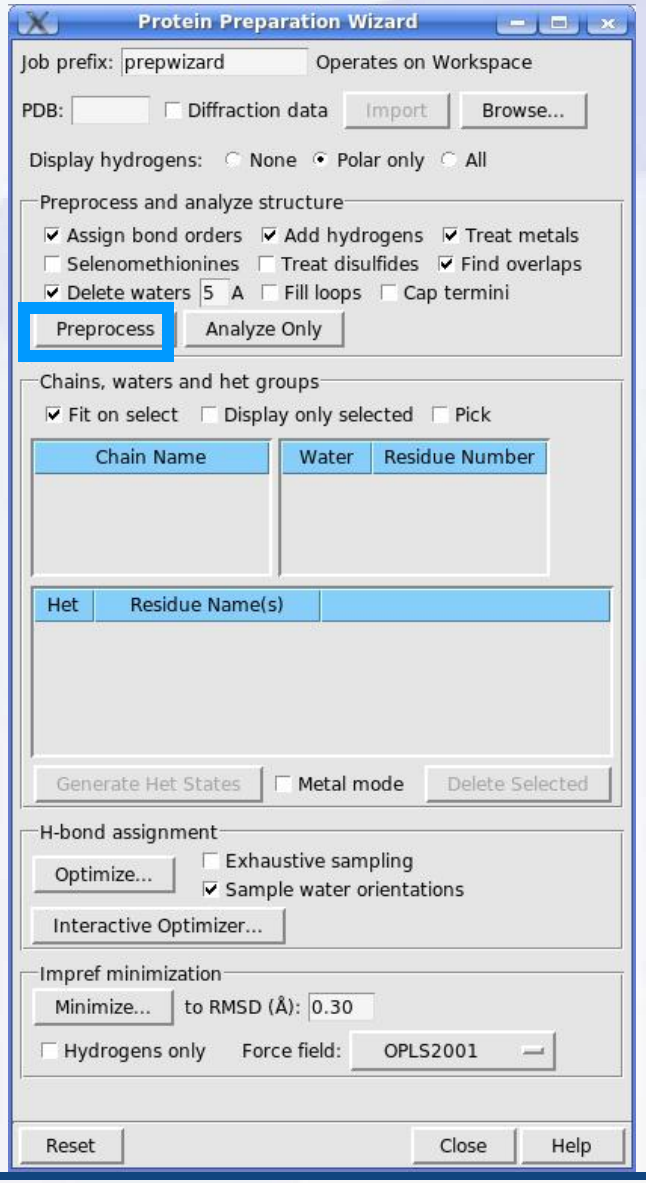


Prepare for simulation: Prepare protein

Check these options:

- Assign bond orders
- Add hydrogens
- Cap termini
  (to cap N or C termini)
- Treat metals
- Treat disulfides
- Find overlaps
- Delete waters

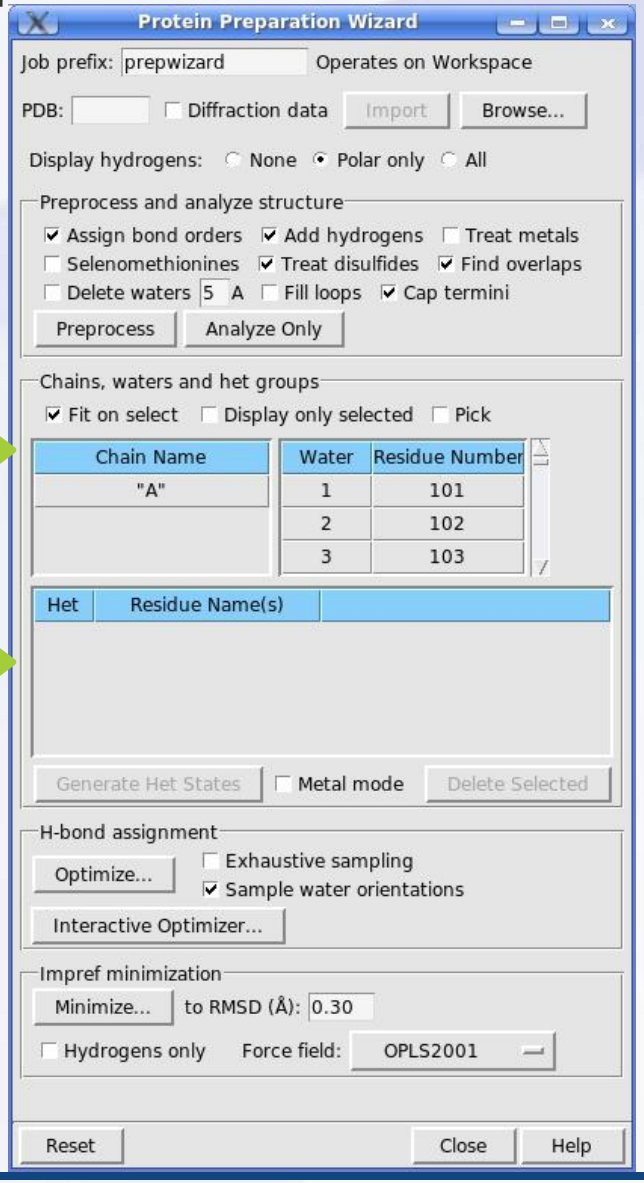
Click Preprocess



Prepare for simulation: Prepare protein

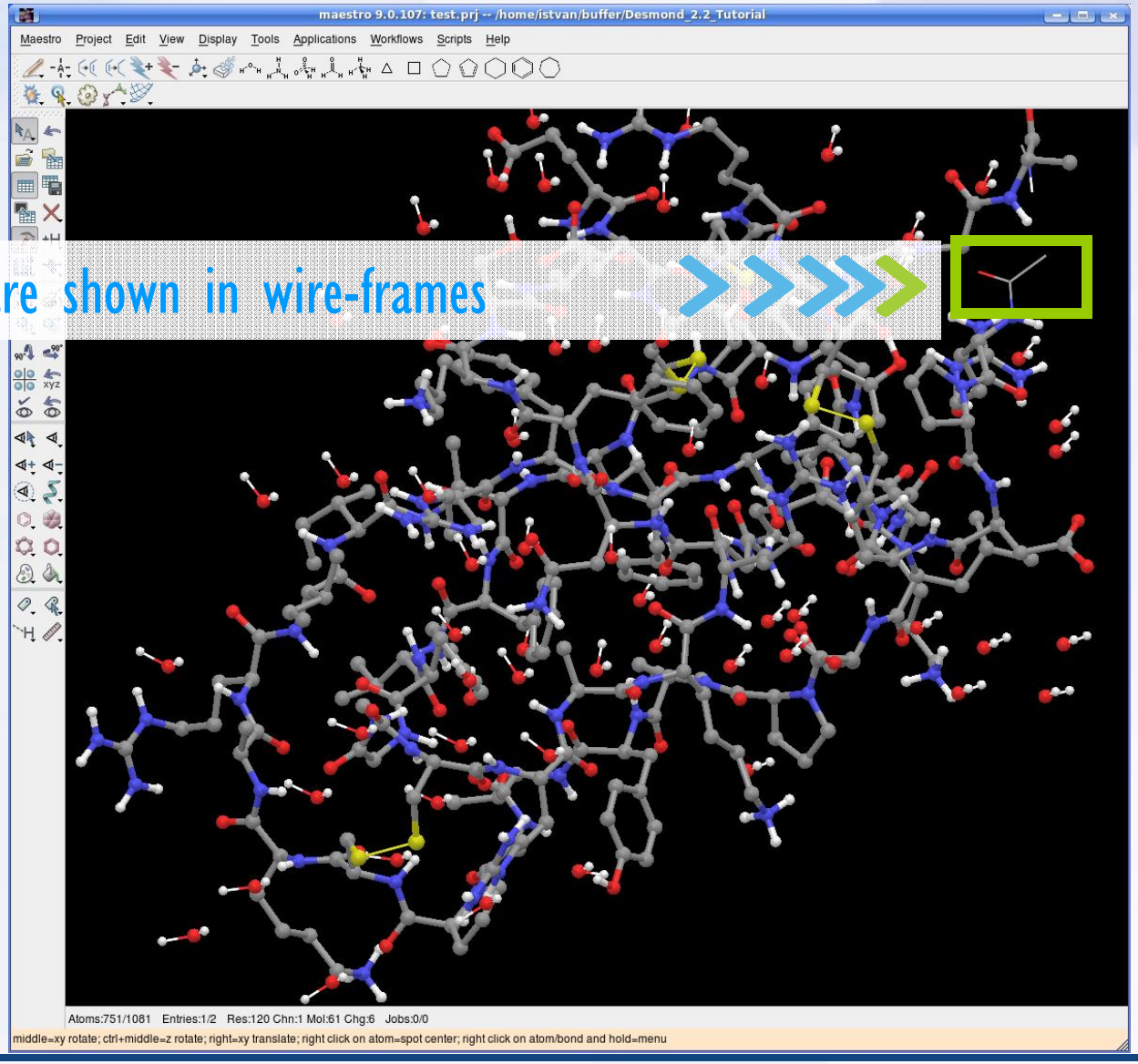
Residue chains and water molecules are listed here

HET groups (if any) are listed here



Prepare for simulation: Prepare protein

Added capping groups are shown in wire-frames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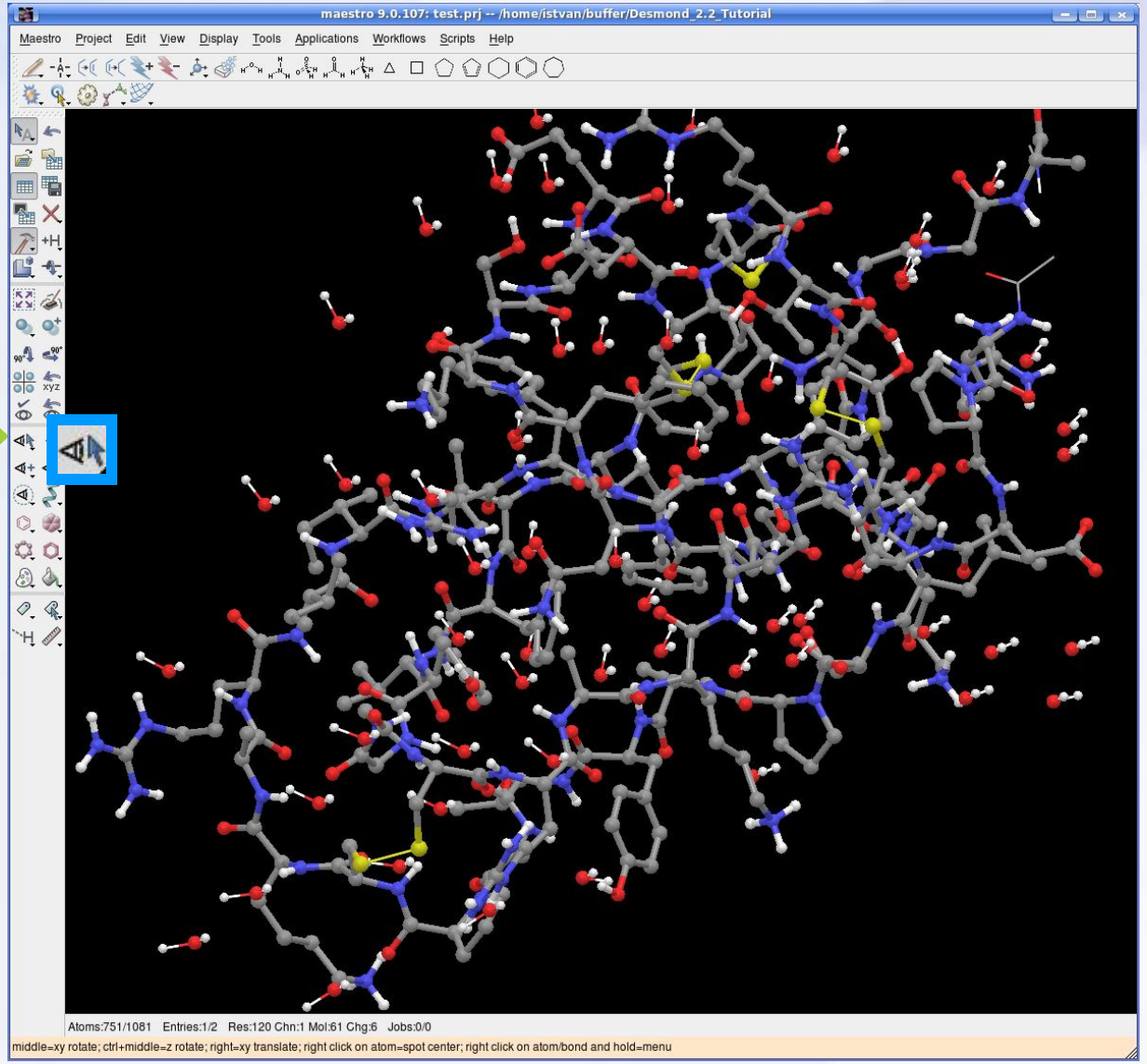
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Prepare for simulation: Prepare protein

Display added hydrogen atoms by double-clicking Display Only Selected Atoms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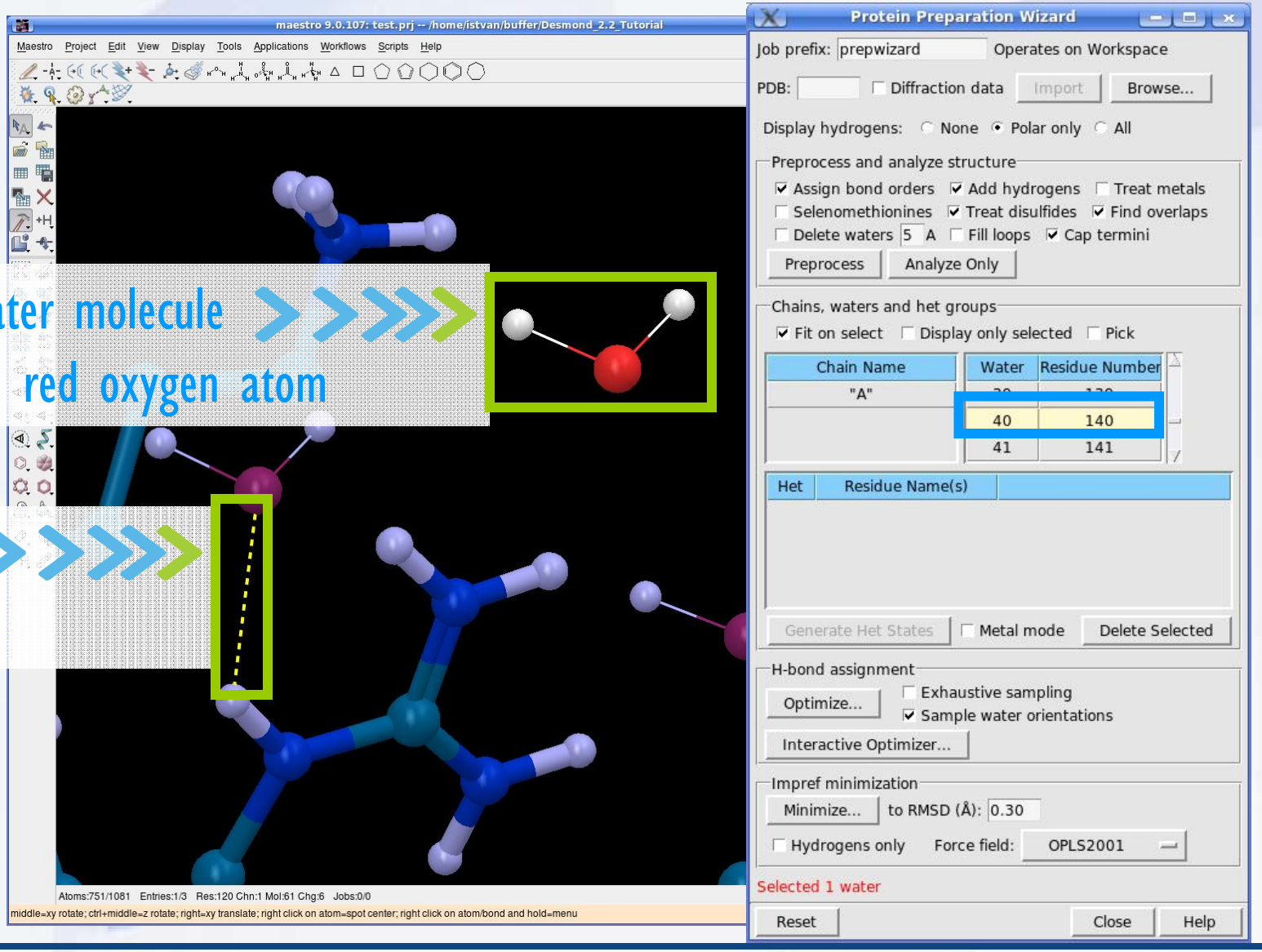
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:751/1081 Entries:1/2 Res:120 Chn:1 Mol:61 Chg:6 Jobs:0/0
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

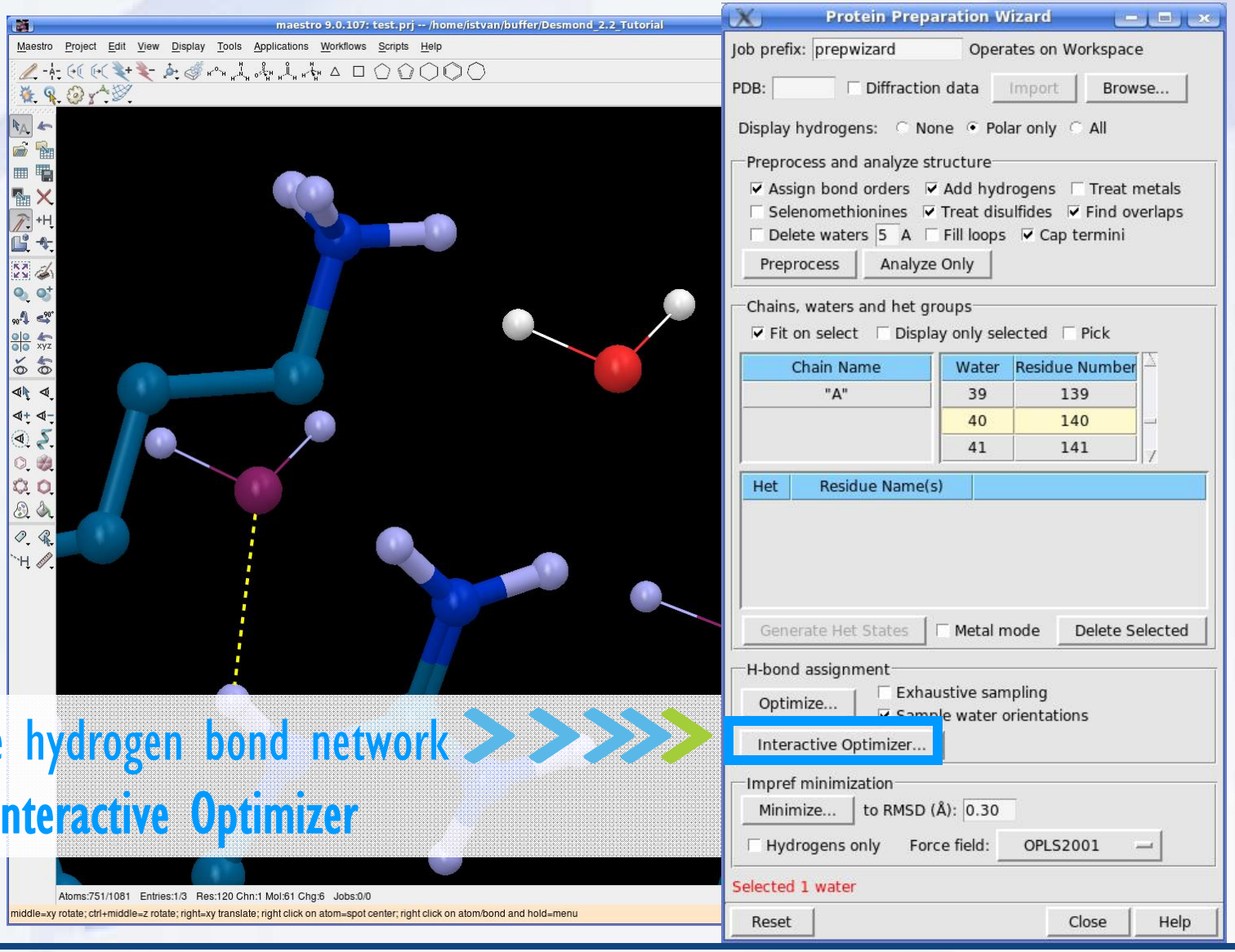
Prepare for simulation: Prepare protein



Selected water molecule
Shown with red oxygen atom

Hydrogen bond

Prepare for simulation: Prepare protein

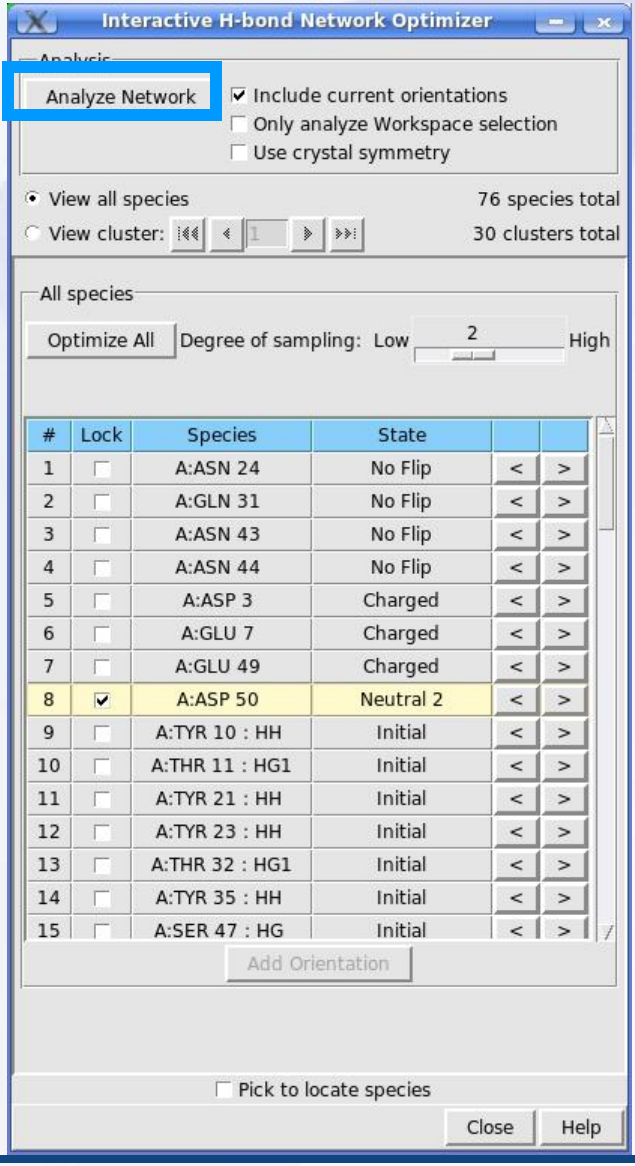


Optimize the hydrogen bond network by clicking **Interactive Optimizer**

Prepare for simulation: Prepare protein

Click **Analyze Network**

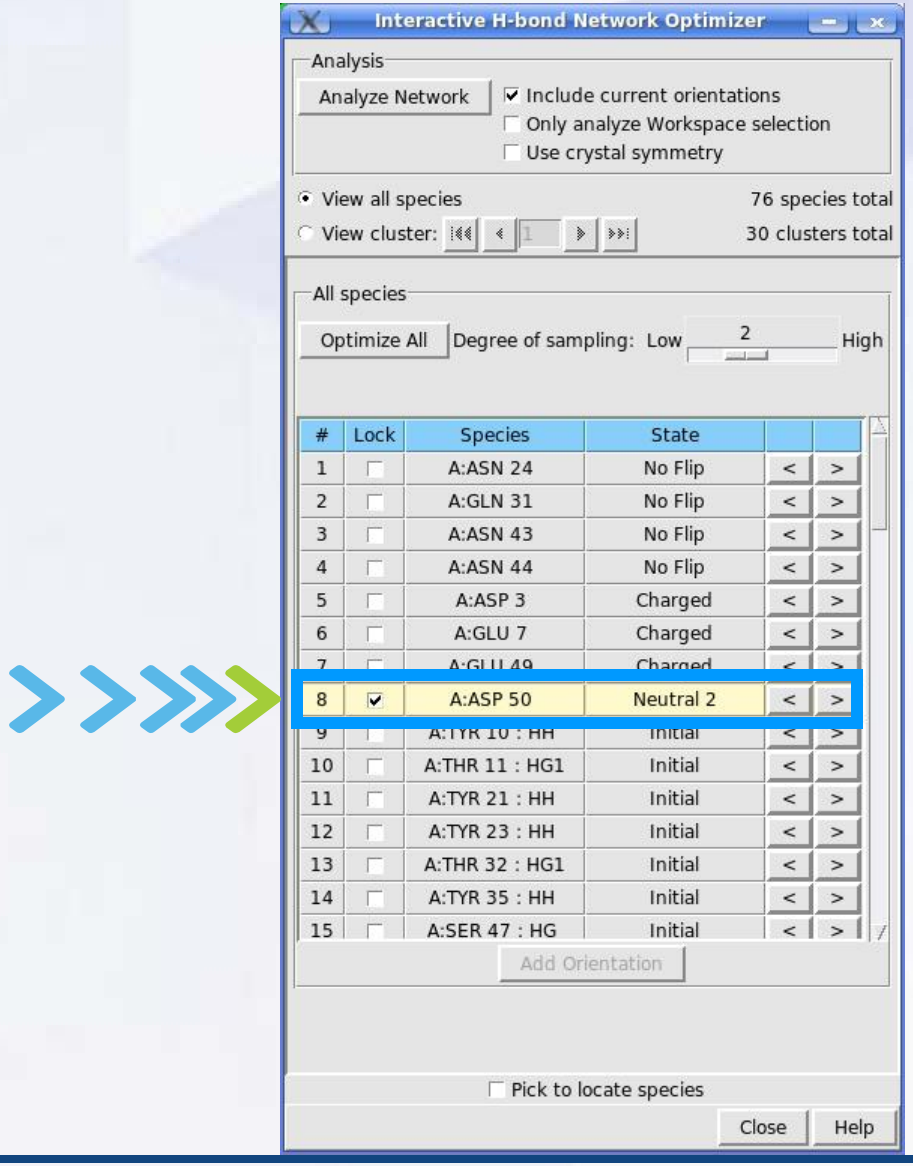
Current protonation states of ASP, GLU and HIS residues are filled in below, as well as OH bond orientations



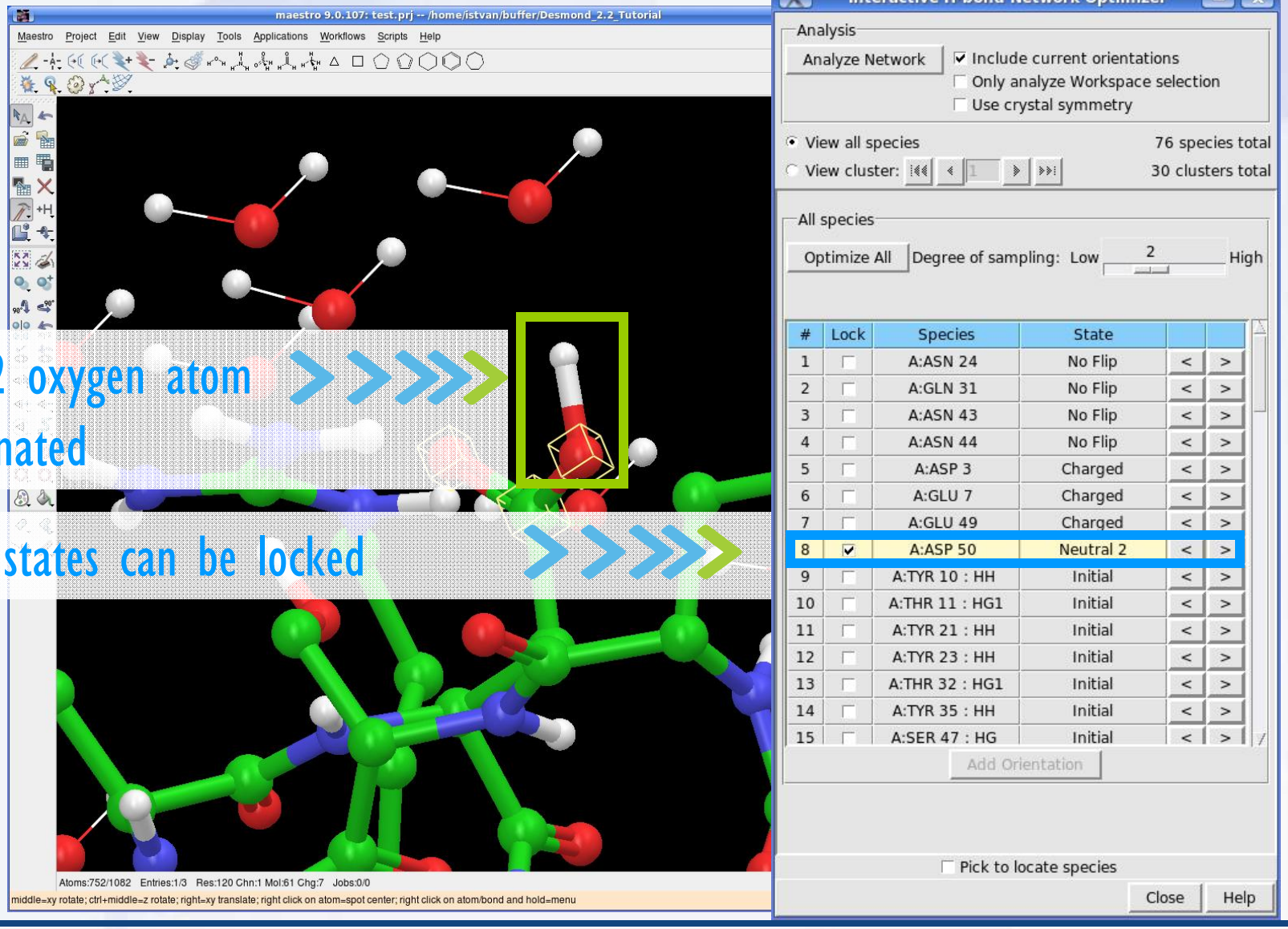
Prepare for simulation: Prepare protein

Select an item to focus the Workspace on a residue

Flip through protonation states with the arrow buttons



Prepare for simulation: Prepare protein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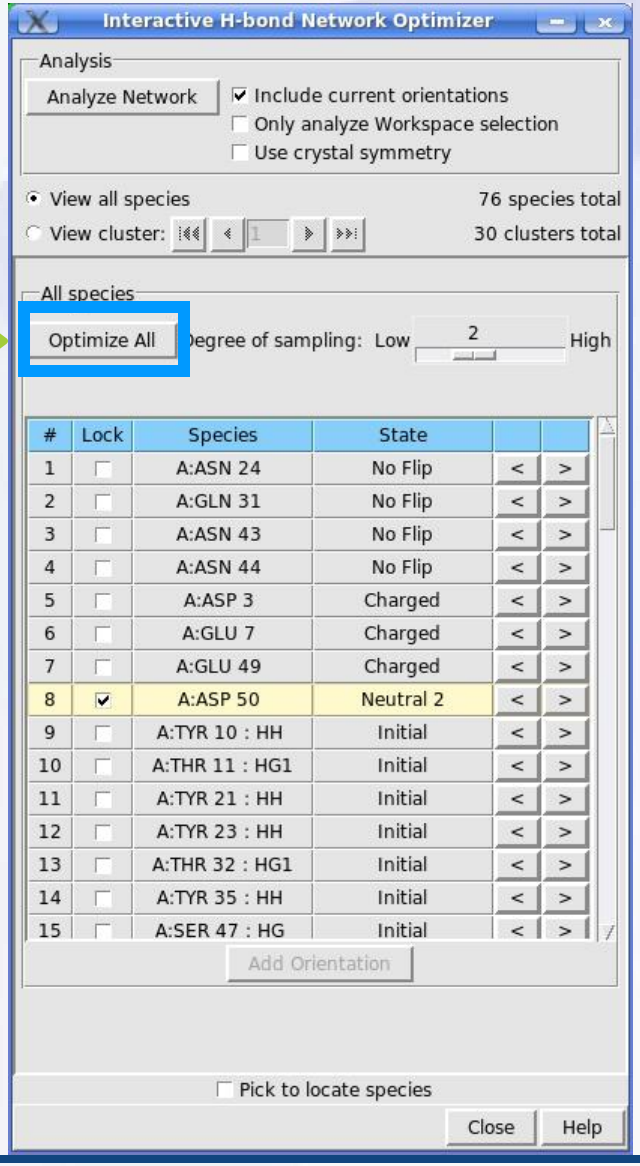
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:752/1082 Entries:1/3 Res:120 Chn:1 Mol:61 Chg:7 Jobs:0/0
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu
Interactive H-bond Network Optimizer
Analysis
Analyze Network
Include current orientations
Only analyze Workspace selection
Use crystal symmetry
View all species
76 species total
View cluster:
30 clusters total
All species
Optimize All
Degree of sampling: Low 2 High
Lock Species State
1 A:ASN 24 No Flip
2 A:GLN 31 No Flip
3 A:ASN 43 No Flip
4 A:ASN 44 No Flip
5 A:ASP 3 Charged
6 A:GLU 7 Charged
7 A:GLU 49 Charged
8 A:ASP 50 Neutral 2
9 A:TYR 10 : HH Initial
10 A:THR 11 : HG1 Initial
11 A:TYR 21 : HH Initial
12 A:TYR 23 : HH Initial
13 A:THR 32 : HG1 Initial
14 A:TYR 35 : HH Initial
15 A:SER 47 : HG Initial
Add Orientation
Pick to locate species
Close
Help

The OD2 oxygen atom is protonated

Residue states can be locked

Prepare for simulation: Prepare protein

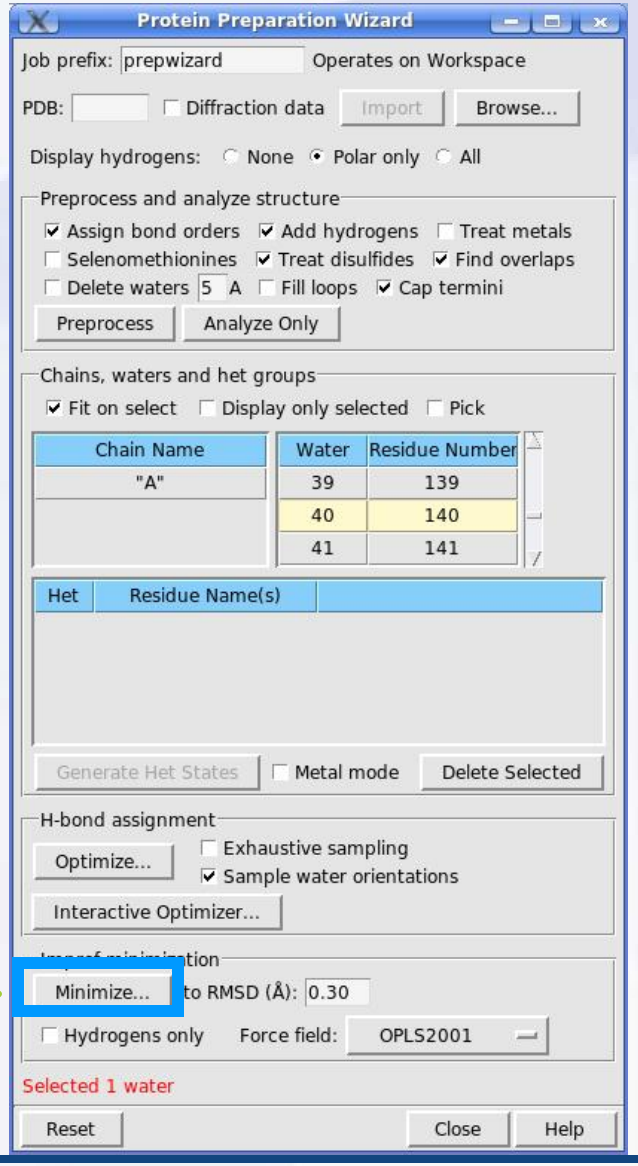
Click **Optimize All** to re-optimize the hydrogen bonding network



Refine the protein structure

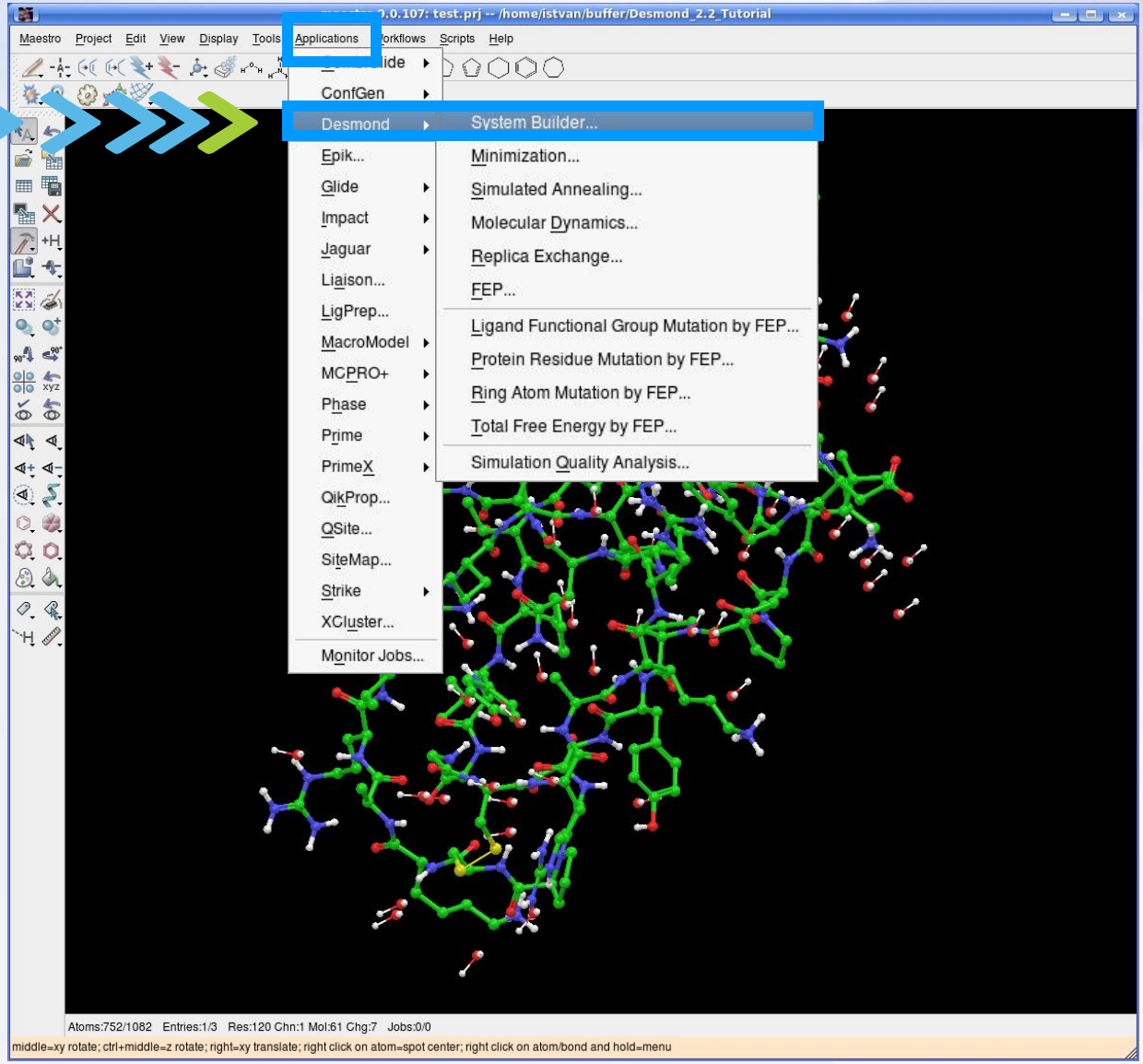
- Subject the protein to constrained refinement
- Click **Minimize** (requires Glide license)
- Preferred method is using Desmond's own minimization protocol

Click the **Minimize** button



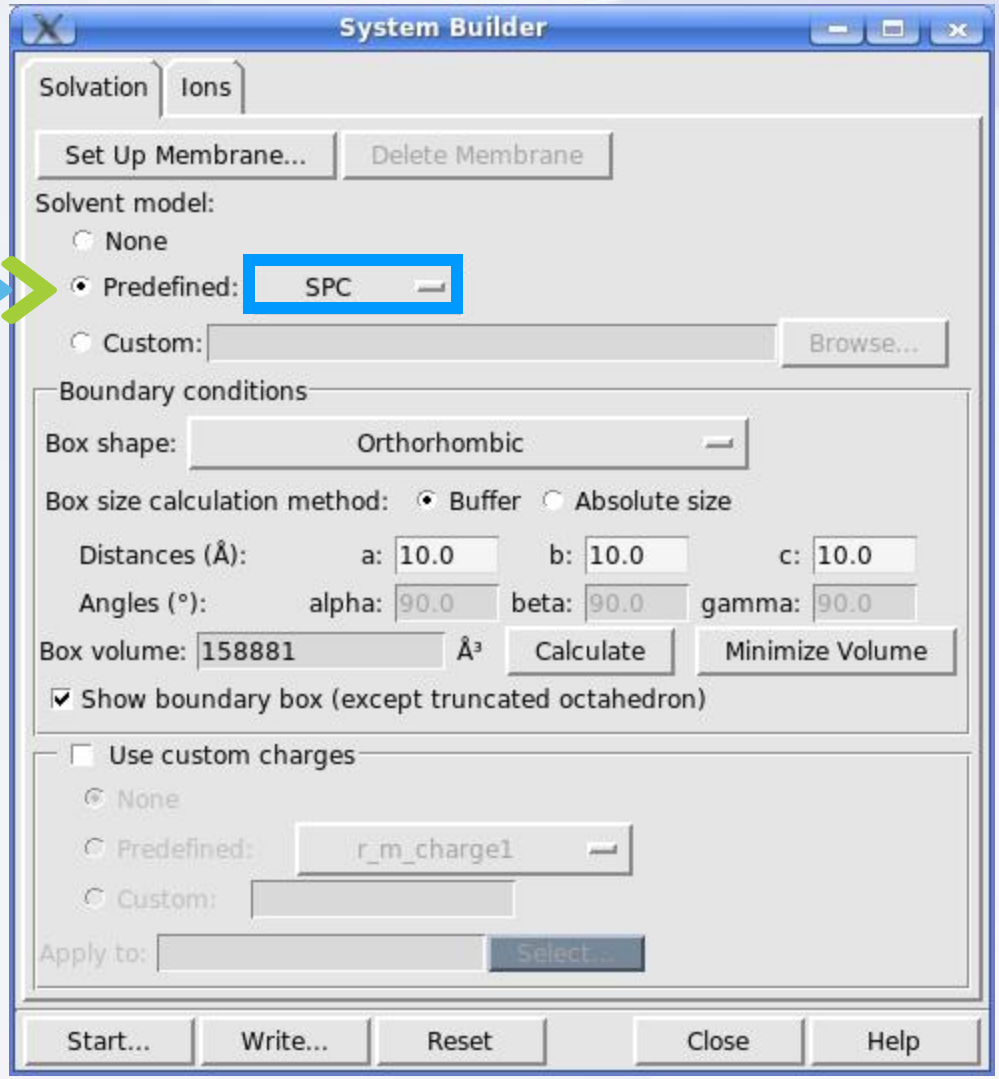
Generate a solvated system

Select Applications > Desmond > System Builder



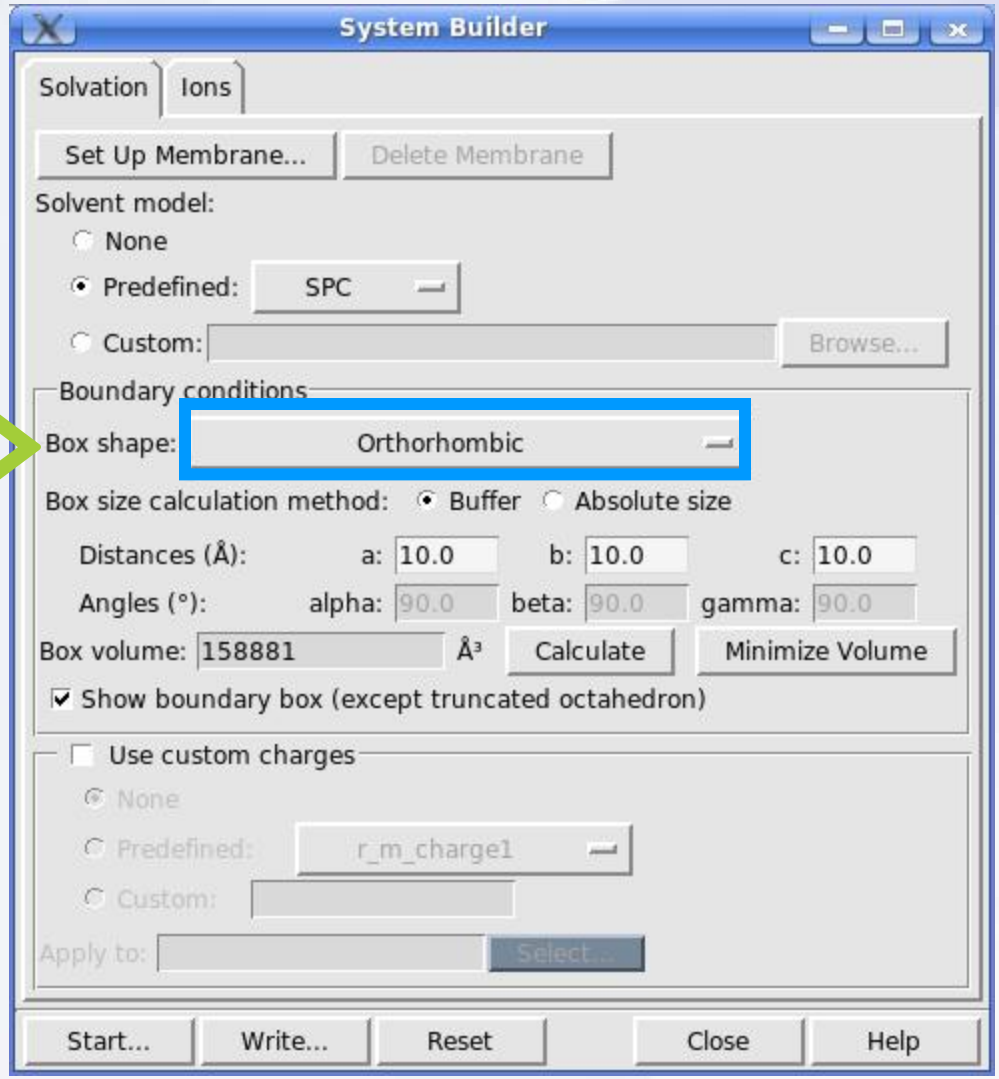
Generate a solvated system

Select SPC as the solvent model



Generate a solvated system

Select **Orthorhombic** as the shape of the solvent box



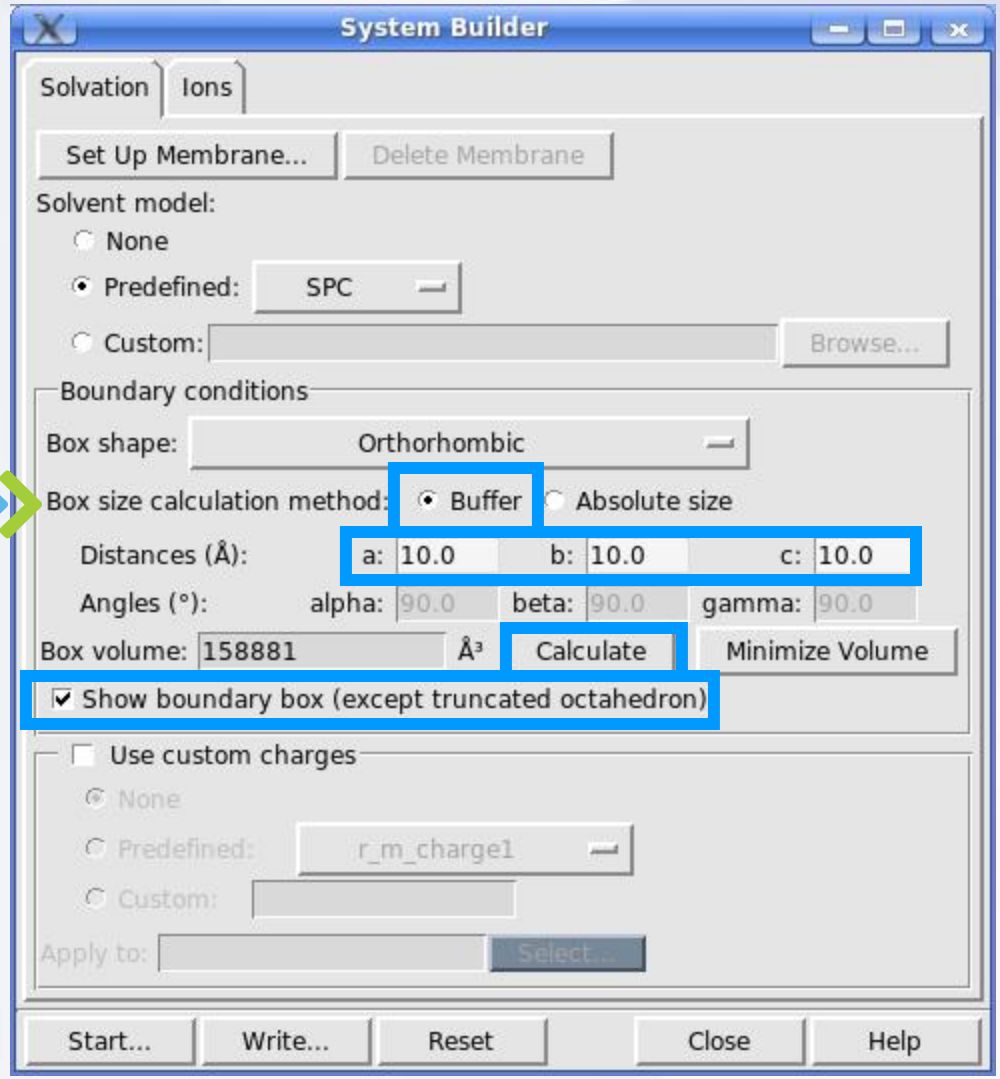
Generate a solvated system

Select **Buffer** as the method of calculating the size of the solvent box

Set the **Distances** to 10 Å

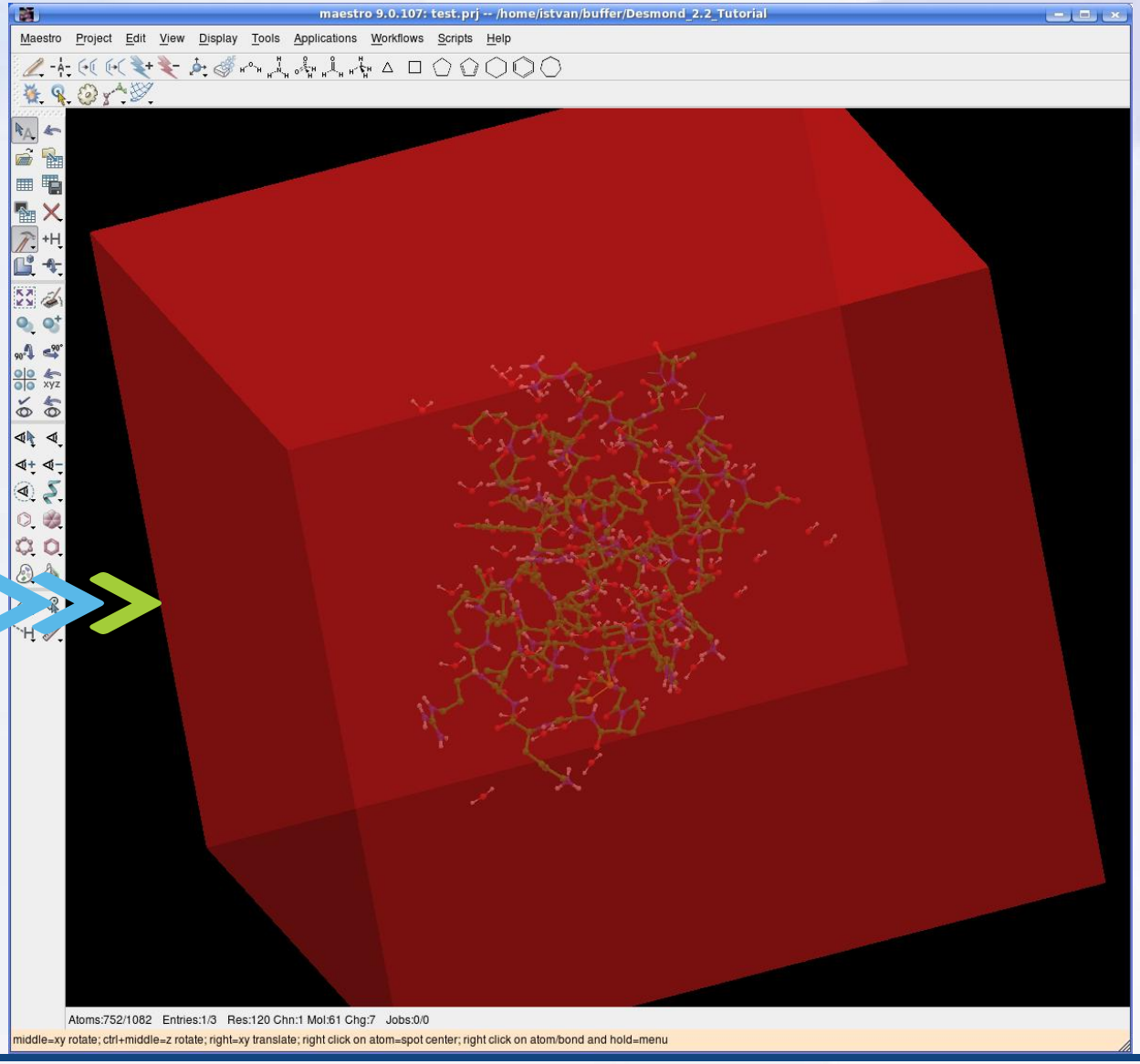
Select **Show boundary box**

Click **Calculate**



Generate a solvated system

The solute and boundary box


maestro 9.0.107: test.prj -- /home/istvan/buffer/Desmond_2.2_Tutorial
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:752/1082 Entries:1/3 Res:120 Chn:1 Mol:61 Chg:7 Jobs:0/0
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

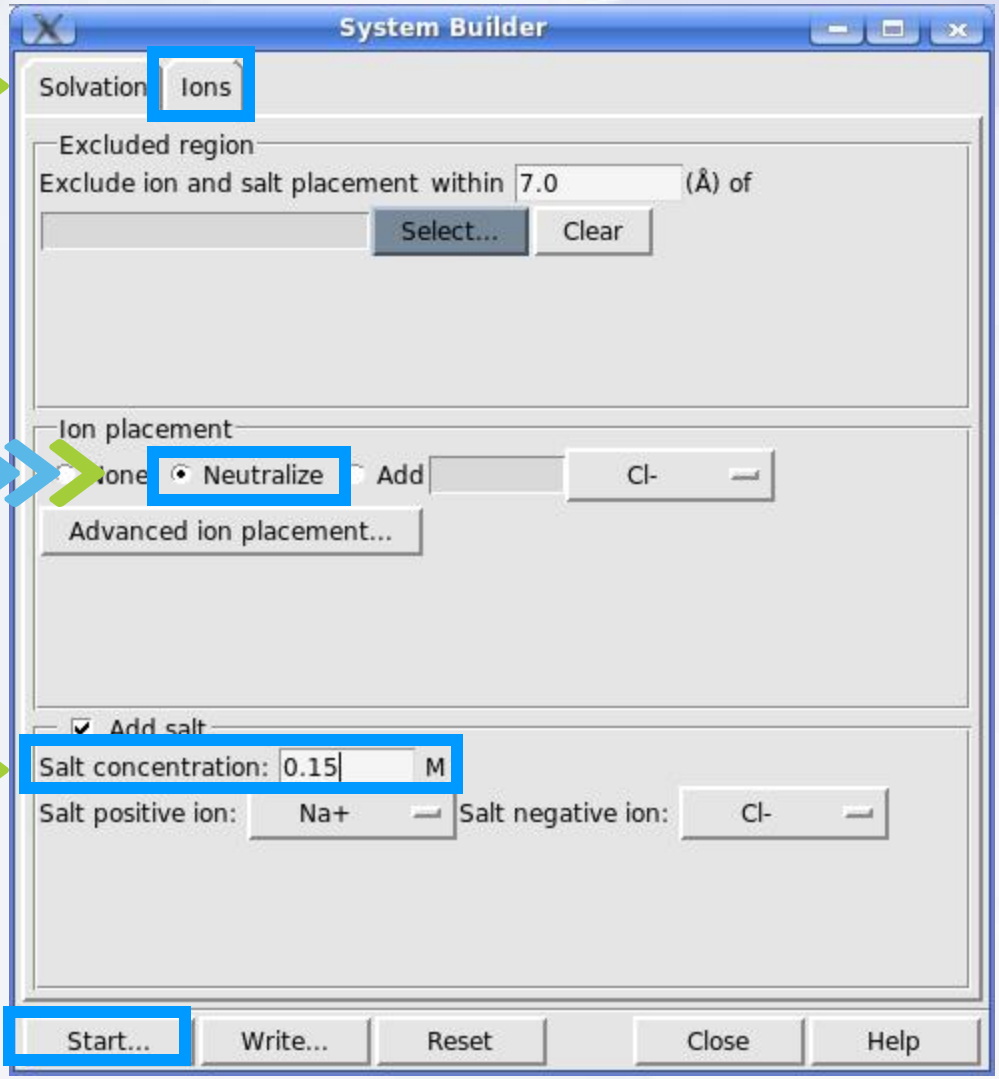
Neutralize the system

Click the **Ions** tab

Select the **Neutralize** option

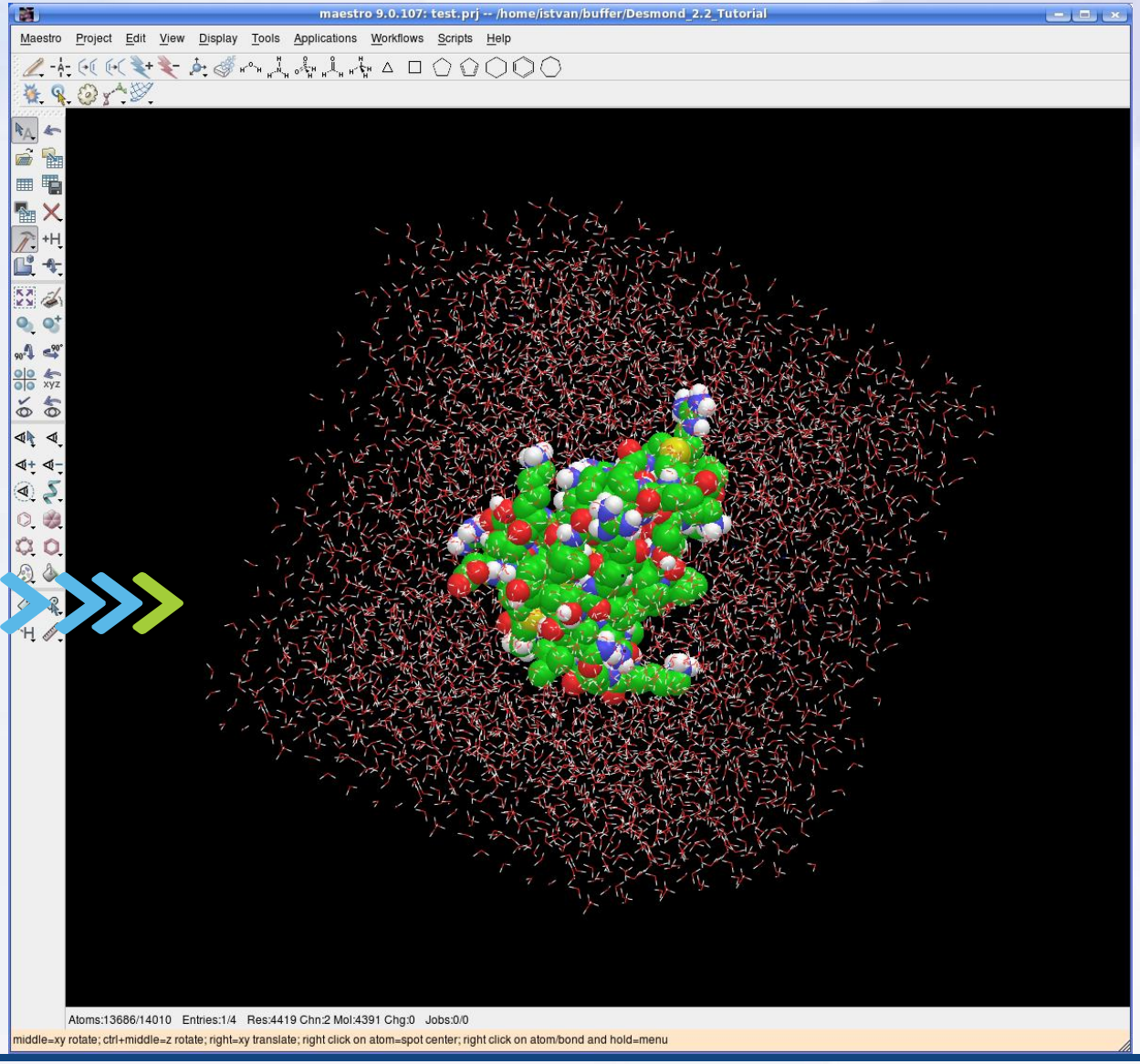
Enter 0.15 M salt concentration

Click **Start**



Generate a solvated system

The solvated system is shown as a CPK model for clarity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Maestro Project Edit View Display Tools Applications Workflows Scripts Help
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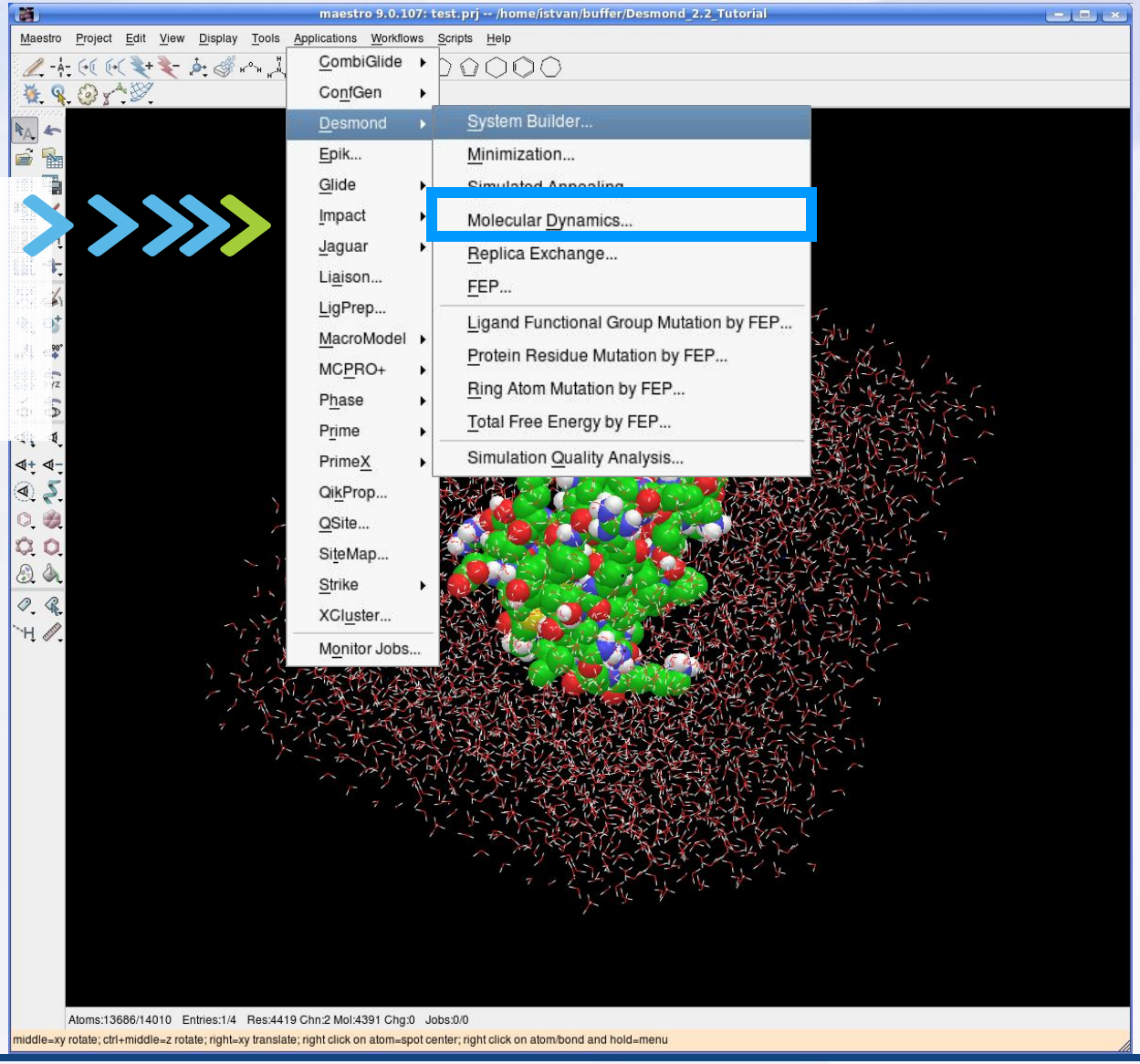
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Assign force field parameters

- Maestro automatically assigns the latest OPLS-AA force field parameters to the system
- Desmond offers additional force fields that can be applied with Viparr

Configure simulation parameters

Select Applications > Desmond > Molecular Dynamics



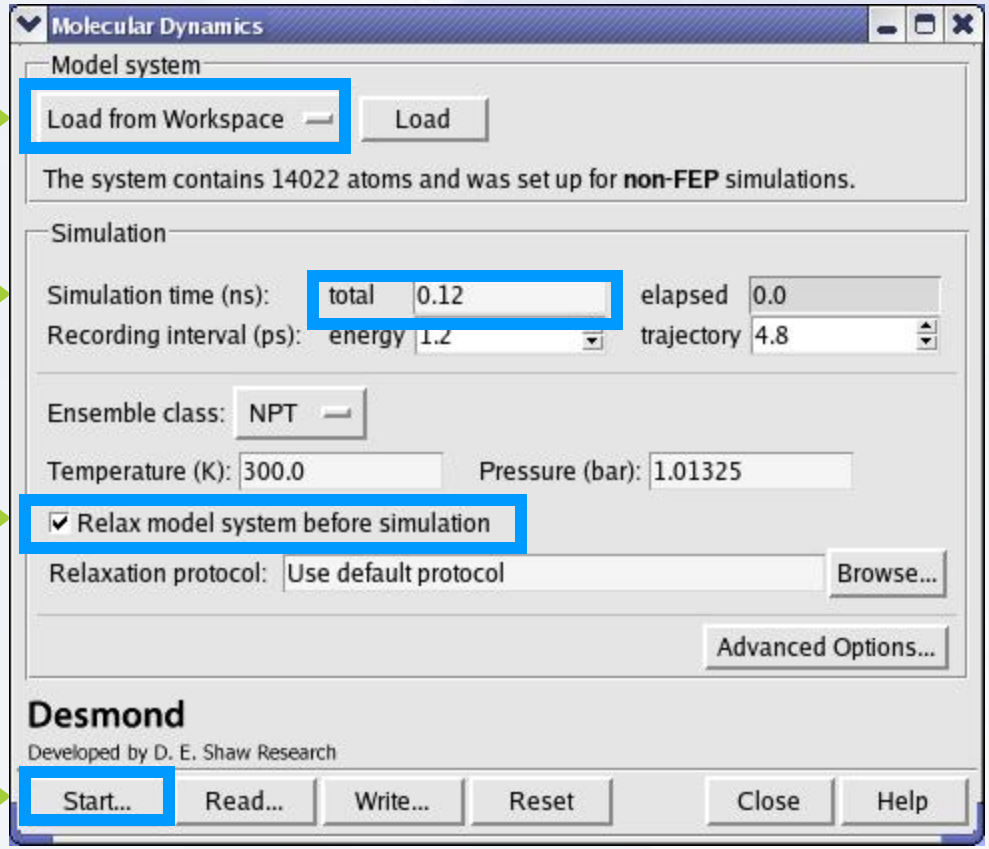
Configure simulation parameters

Select **Load from Workspace**

Enter 0.12 ns as the simulation time

Select the **Relax model system before simulation** option

Click **Start**



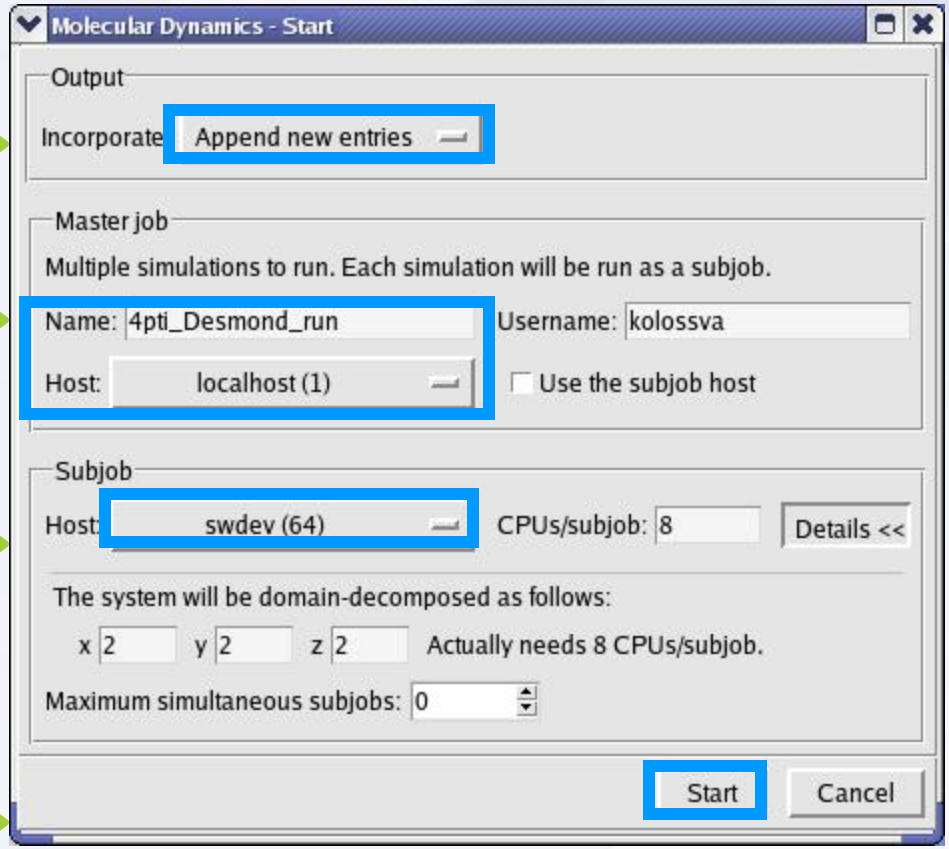
Configure job submission options

Select **Append new entries**

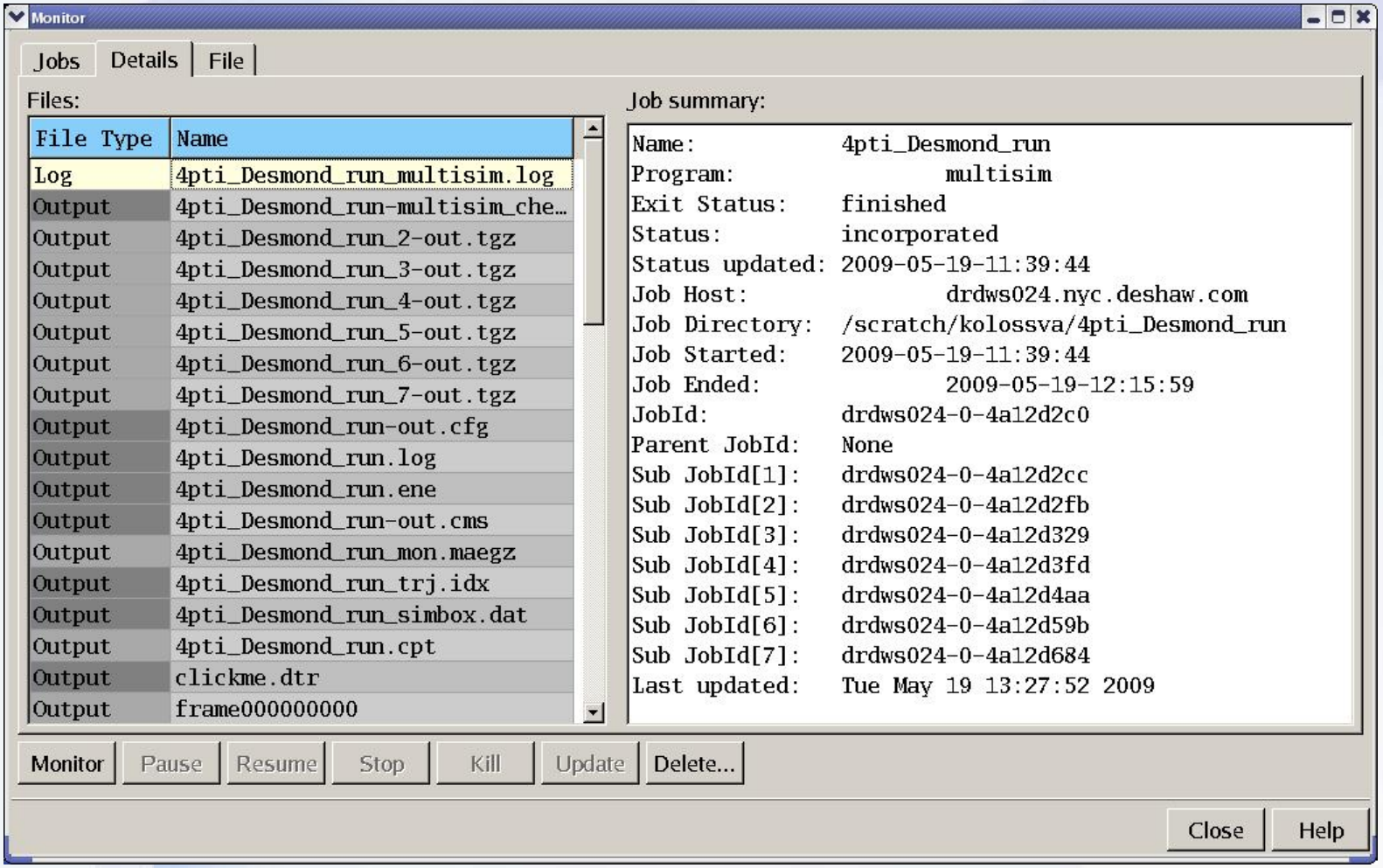
Enter a name for the simulation and select the **Master** host

Select the subjob **Host** and run options

Click **Start**

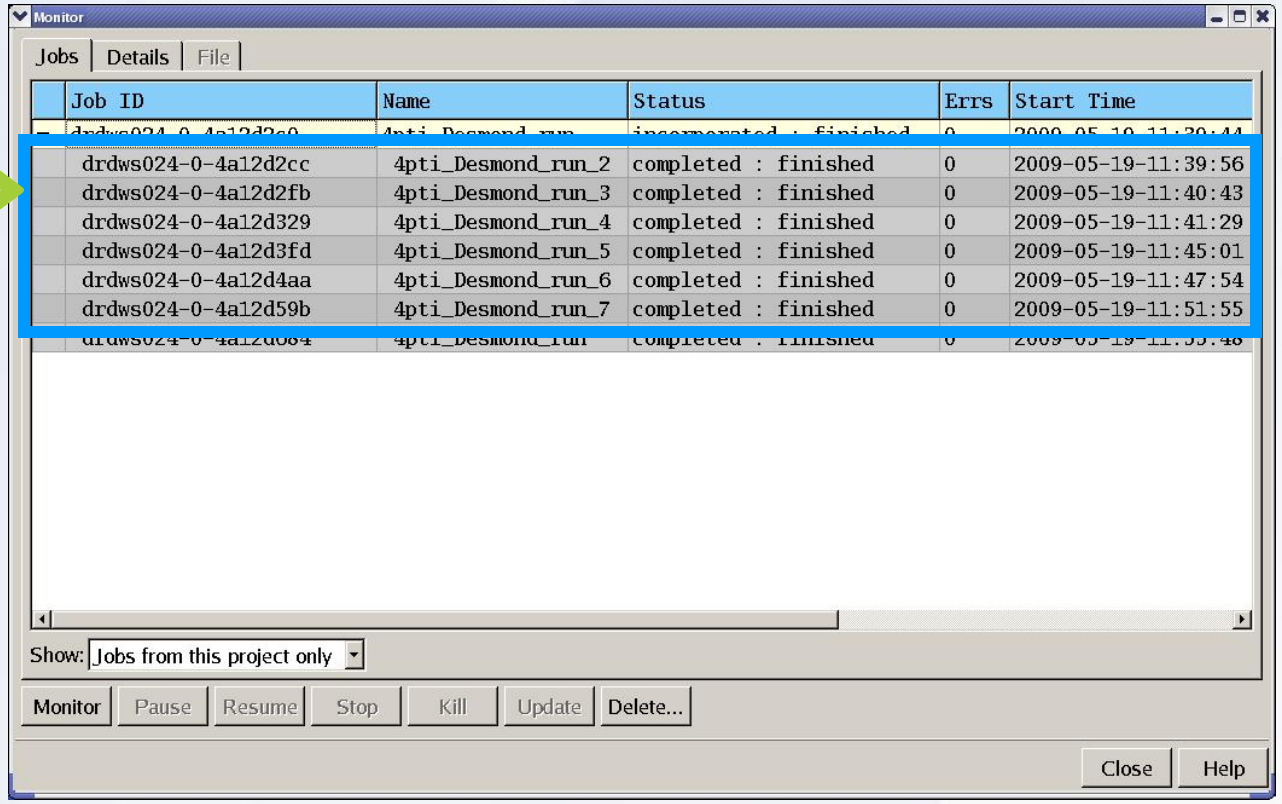


Monitor the simulation



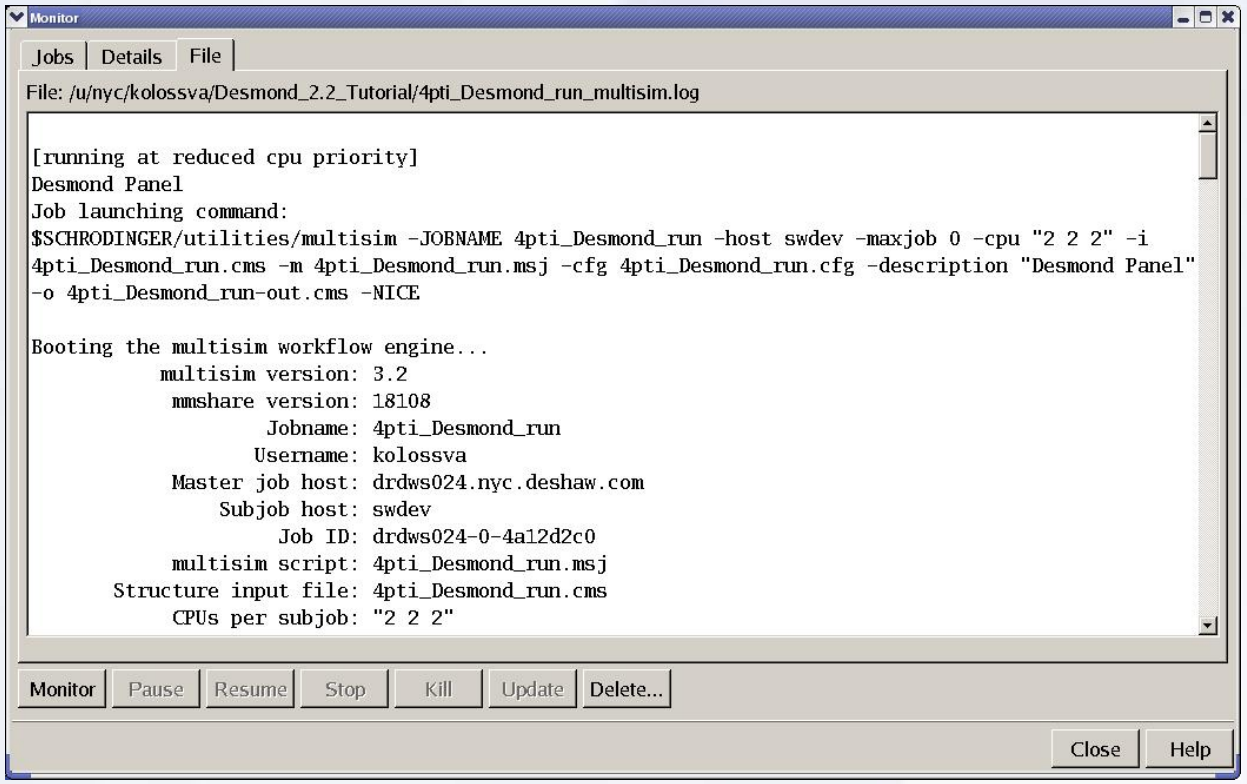
Monitor the simulation

Jobs 2-7 are the relaxation phase



Monitor the simulation

List of files created by the job

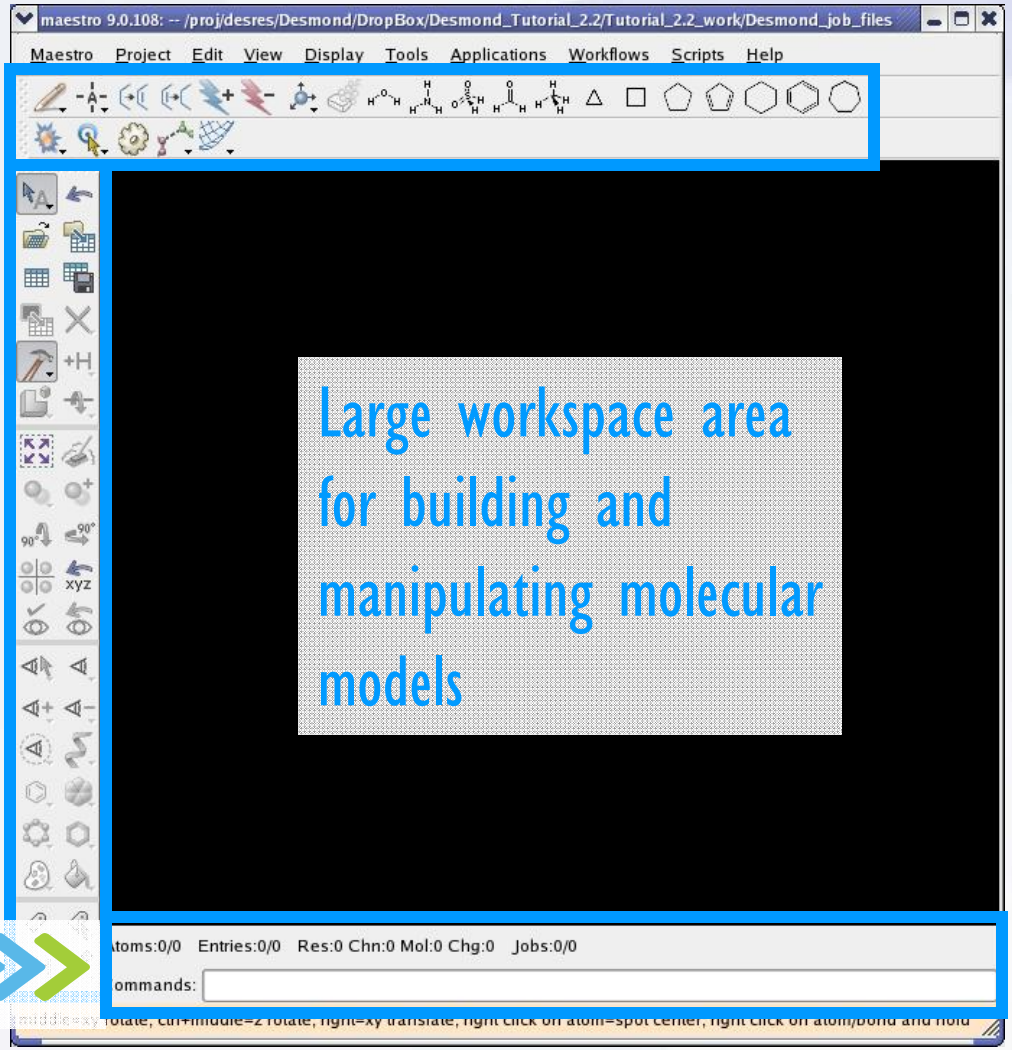


The Maestro environment

Horizontal toolbar

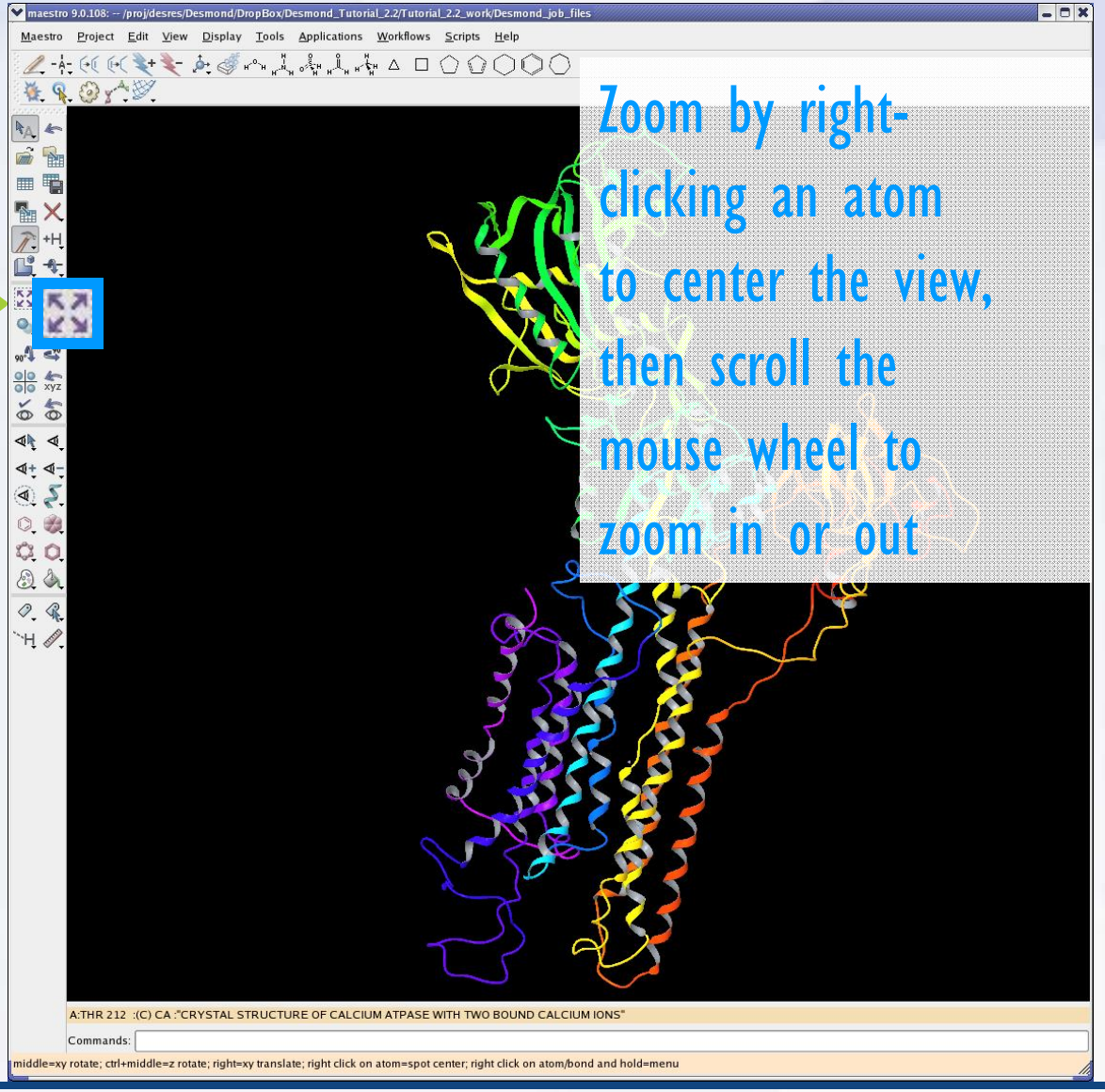
Vertical toolbar

Command line


maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Large workspace area for building and manipulating molecular models
toms:0/0 Entries:0/0 Res:0 Chn:0 Mol:0 Chg:0 Jobs:0/0
ommands:

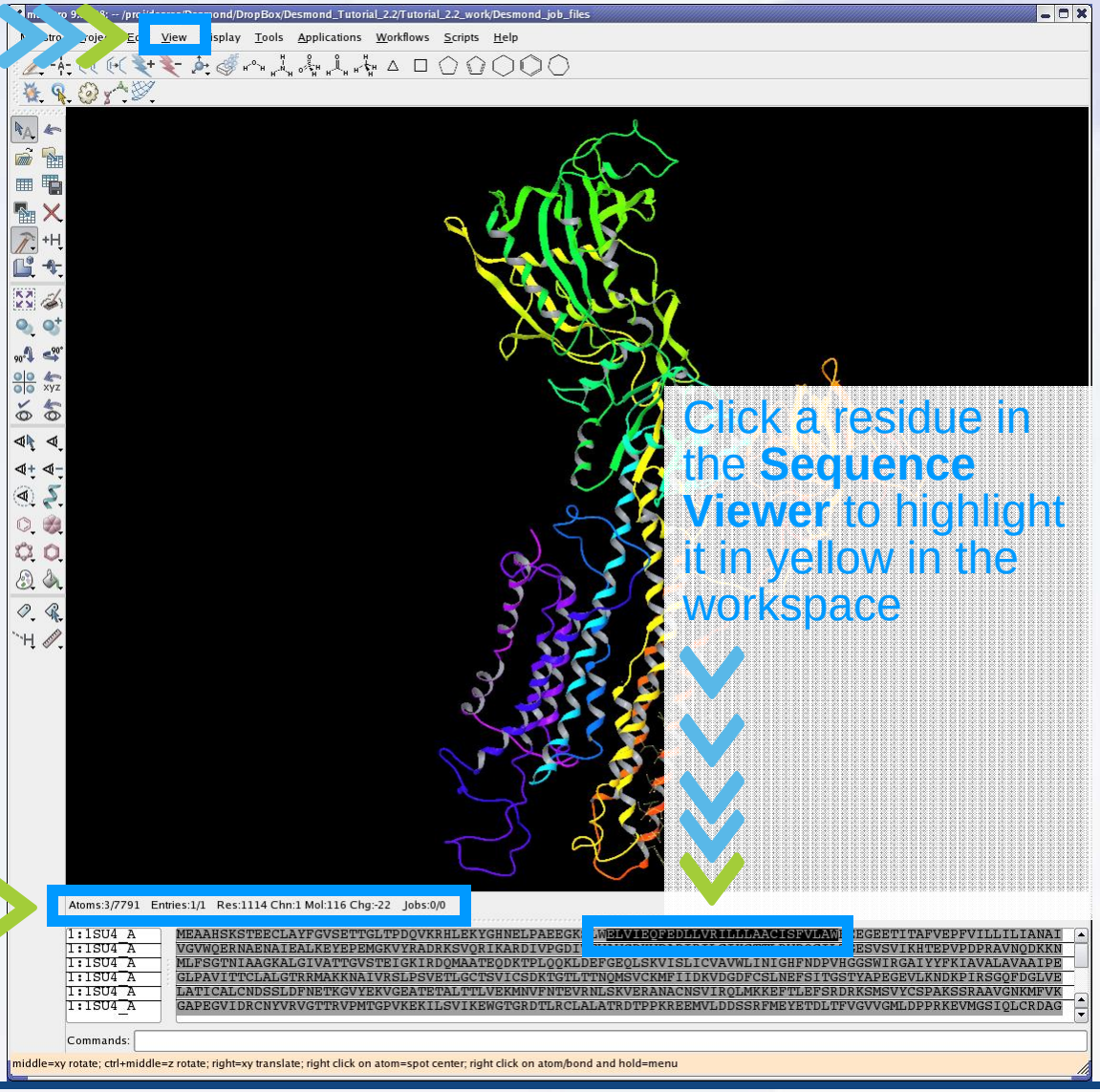
Zooming in & Restoring full view

Click the **Fit to Screen** button to show the full size view of the molecule


maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Zoom by right-clicking an atom to center the view, then scroll the mouse wheel to zoom in or out
A:THR 212 :(C) CA :"CRYSTAL STRUCTURE OF CALCIUM ATPASE WITH TWO BOUND CALCIUM IONS"
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Displaying residue and atom information

Select View>Sequence Viewer


Click a residue in the **Sequence Viewer** to highlight it in yellow in the workspace
Atoms:3/7791 Entries:1/1 Res:1114 Chn:1 Mol:116 Chg:-22 Jobs:0/0
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

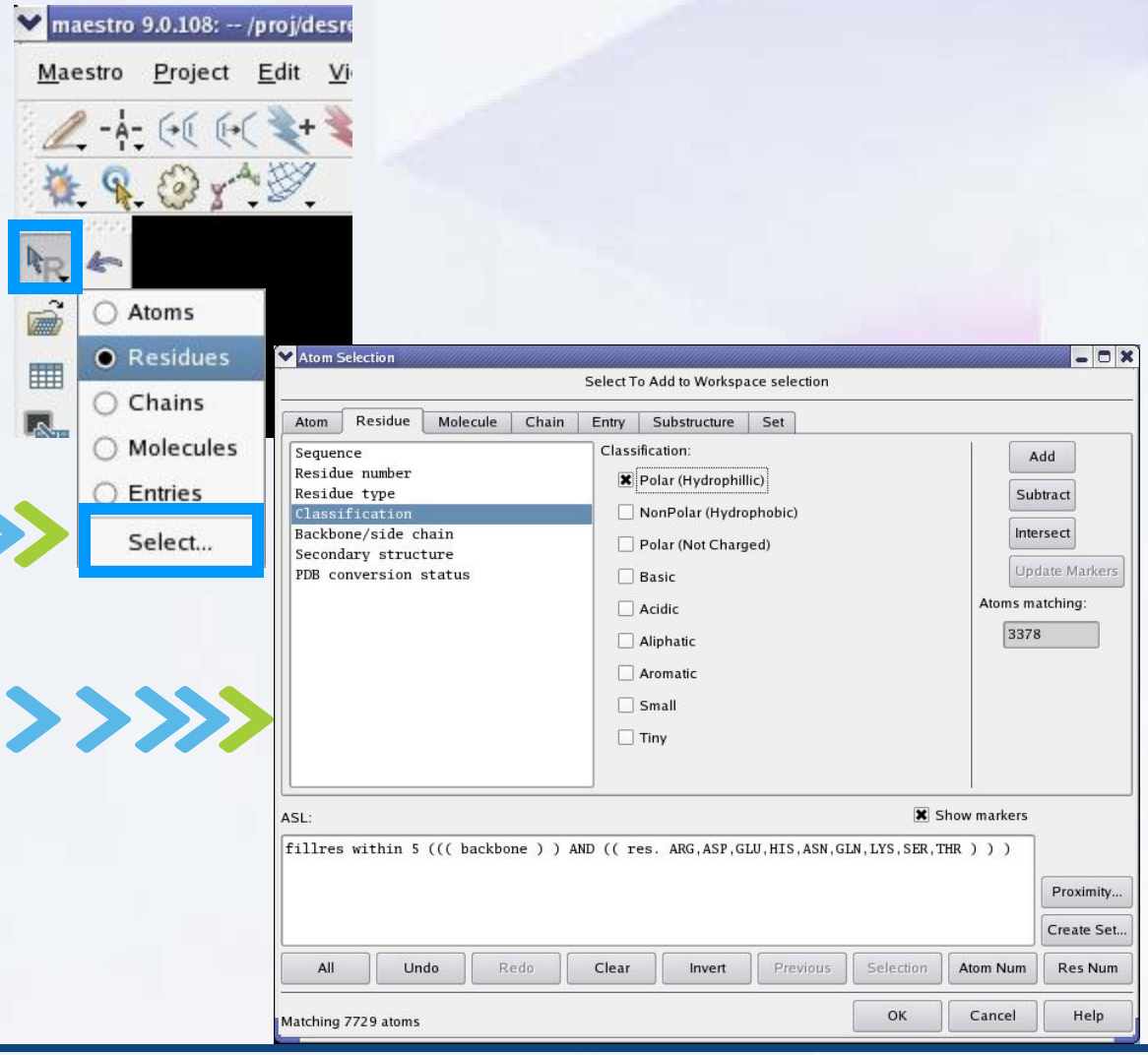
Move the cursor over an atom in the workspace to view its details in the Sequence Viewer

Changing selection mode

Click and hold the **Select** button and make a choice

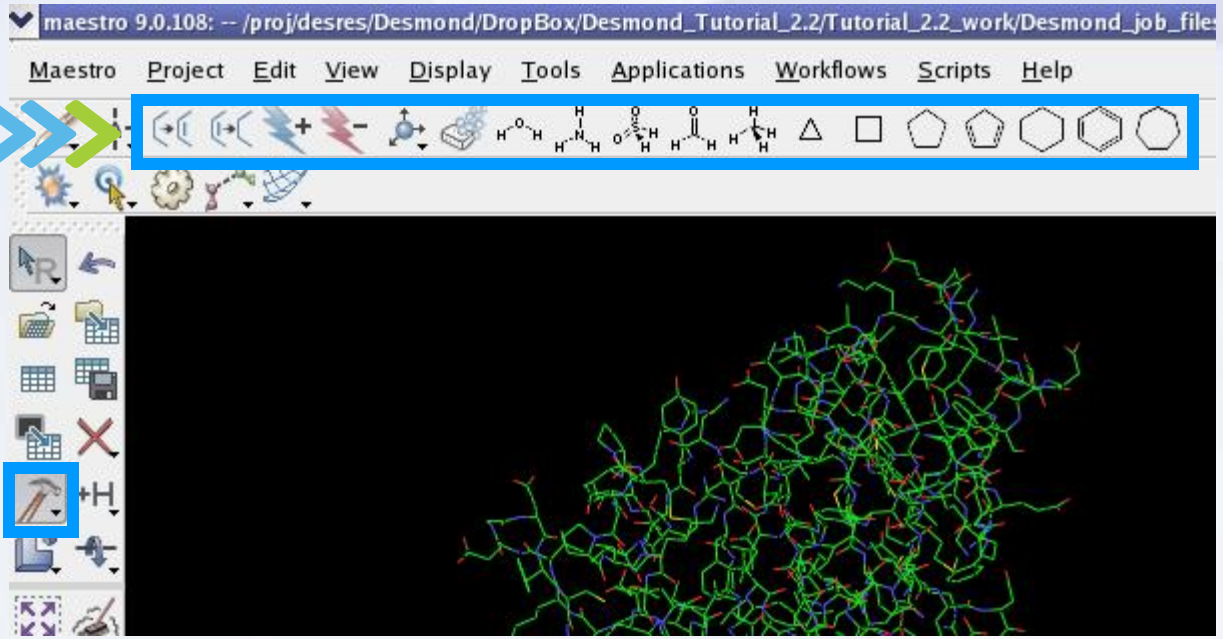
To select arbitrary atoms, choose **Select**

…then make complex selections on the tabs (including SMARTS) and convert selections to ASL



Editing a molecular structure

Tools for building & editing structures appear when you click the **Hammer**

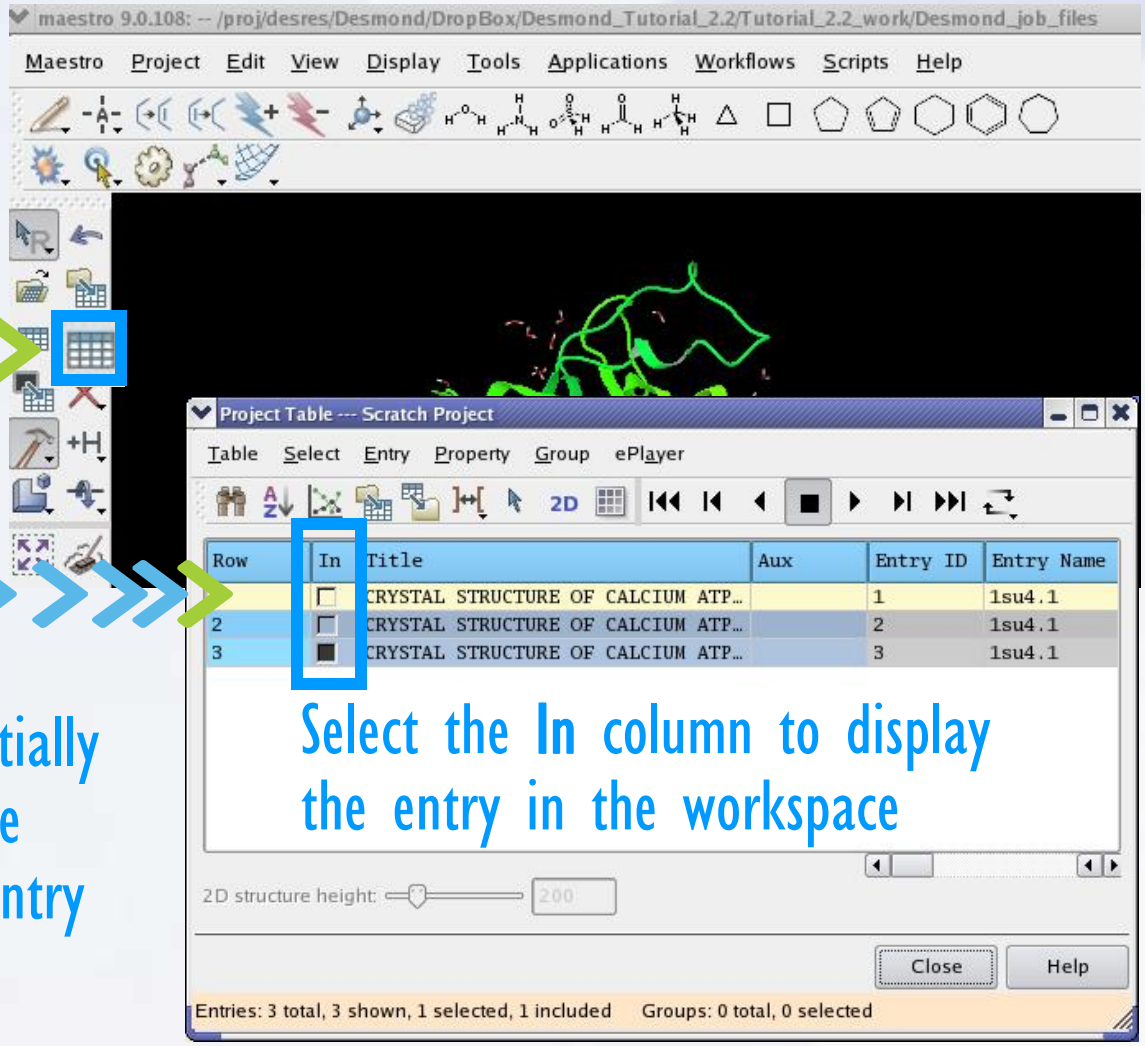


- Hand-draw structures
- Set chemical elements
- Increment/decrement bond order or formal charge
- Adjust the position of atoms
- Clean up 3D geometry
- Add common molecular fragments

Maestro projects

Projects record every change to molecular structures and results of computational tasks

Click the **Project** button


maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Project Table --- Scratch Project
Table Select Entry Property Group ePlayer
2D
Row In Title Aux Entry ID Entry Name
1 CRYSTAL STRUCTURE OF CALCIUM ATP… 1 1su4.1
2 CRYSTAL STRUCTURE OF CALCIUM ATP… 2 1su4.1
3 CRYSTAL STRUCTURE OF CALCIUM ATP… 3 1su4.1
Select the In column to display the entry in the workspace
2D structure height: 200
Close Help
Entries: 3 total, 3 shown, 1 selected, 1 included Groups: 0 total, 0 selected

- The **Project Table** opens
- Each row is an entry
- Entries are created sequentially
- Computational tasks can be applied to the state of an entry selected in the table

Useful tools in Maestro

- **Maestro>Save Image** saves a screen capture of the workspace
- **Project>Make Snapshot** saves the current project state
- **Edit>Clean Up Geometry** generates 3D models of hand-drawn structures
- **Edit>Command Script Editor** enables powerful scripting
- **Display>Surface** generates a variety of molecular surfaces
- **Tools>Sets** defines arbitrary sets of atoms for quick rendering
- **Tools>Protein Structure Alignment** superposes homologue protein structures
- **Tools>Protein Reports** generates useful structural information in a table
- **Applications>Glide** is a versatile fast docking tool
- **Applications>LigPrep** prepares small molecules for simulation
- **Applications>MacroModel** offers molecular mechanics tools
- **Scripts** menu for accessing Maestro functionalities from Python scripts

Handling missing residues or side chains

- Most protein structures in the PDB database have missing residues, or side chains with missing atoms
- Protein Preparation Wizard does not address these issues
- Maestro's Prime tool can automatically build a three-dimensional model of a complete amino acid sequence
- Prime can be 'fooled' into filling in missing residues and side chains in an incomplete protein structure
- You need a license for Prime from Schrödinger, LLC

Running Prime

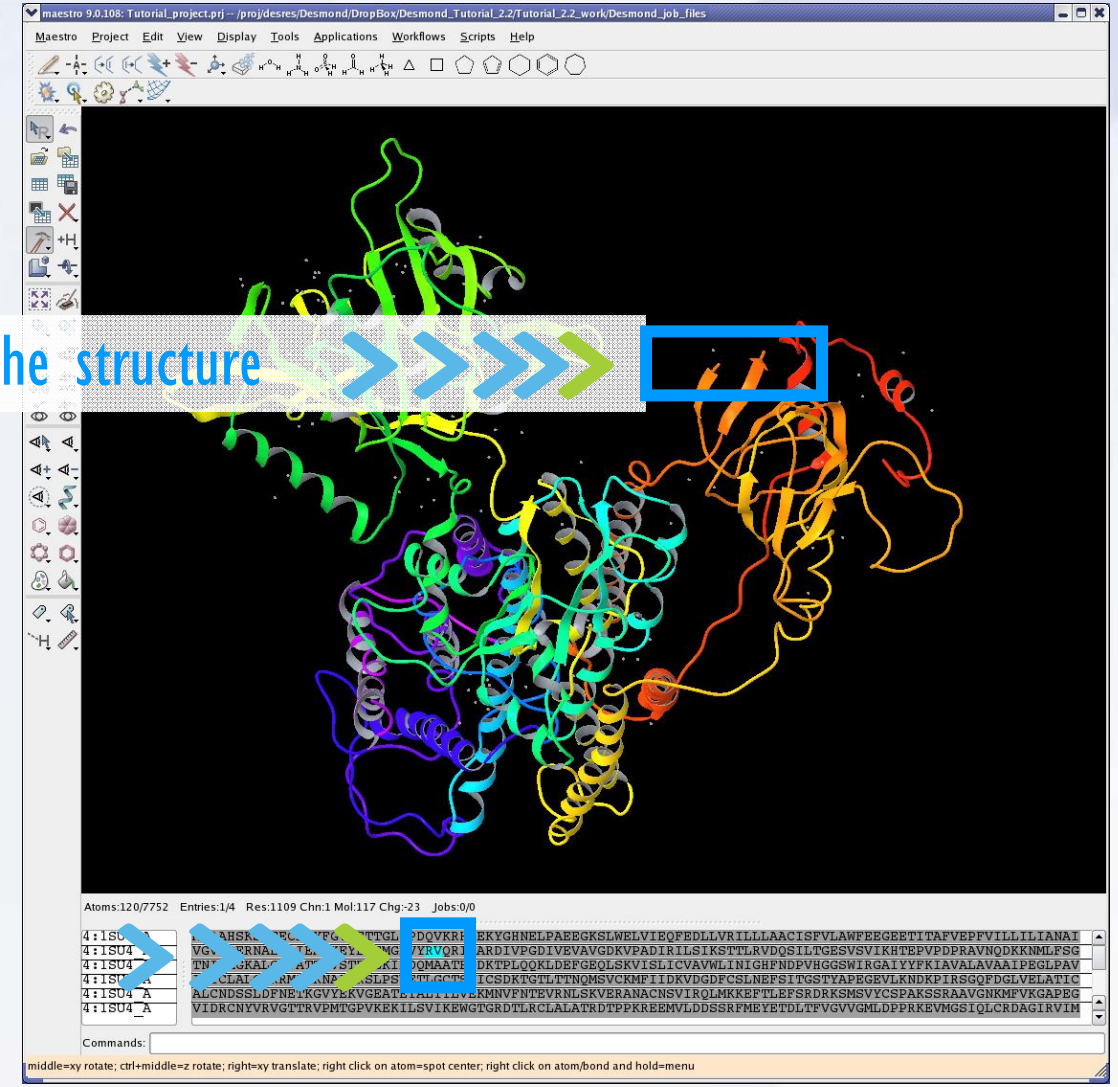
1. Download the 1su4 structure to Maestro
2. Delete residues 132-136 from the structure by hand and save it as **1su4 _missing_loop.pdb**
3. Import the modified PDB structure into Maestro

   Ignore the warning message that refers to the missing residues

Running Prime

The missing loop appears in the structure

Colored residues show the position of the missing loop


maestro 9.0.108: Tutorial_project.prj -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:120/7752 Entries:1/4 Res:1109 Chn:1 Mol:117 Chg:-23 Jobs:0/0
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Running Prime

Apply Prime to the truncated 1su4 protein structure with the missing loop

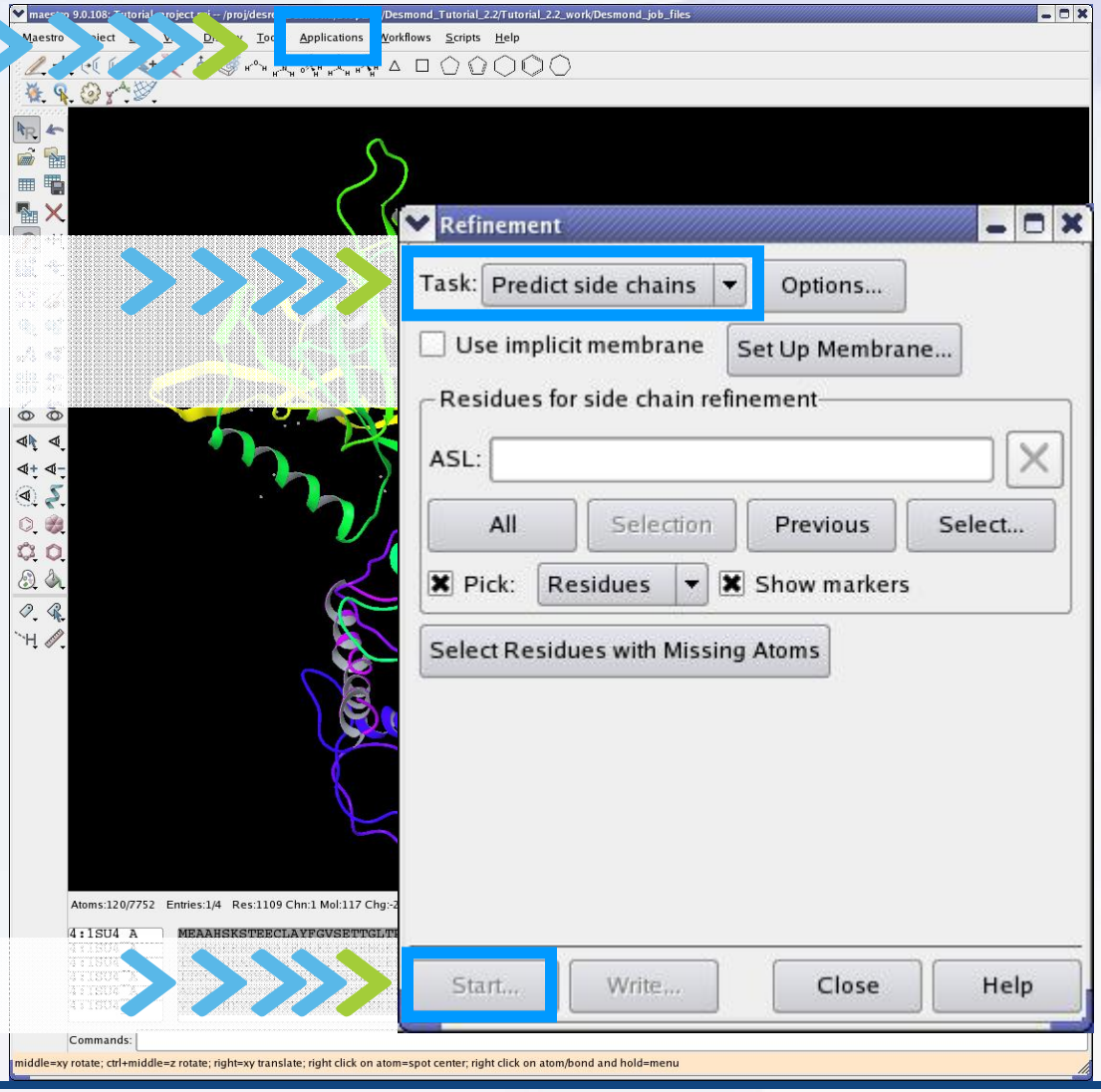
- Simple: Proteins with missing side chains (no missing residues)
- Complex: Proteins with missing residues

Running Prime

Select Applications>Prime> Refinement

Select Predict side chains from the Task field

Click Start



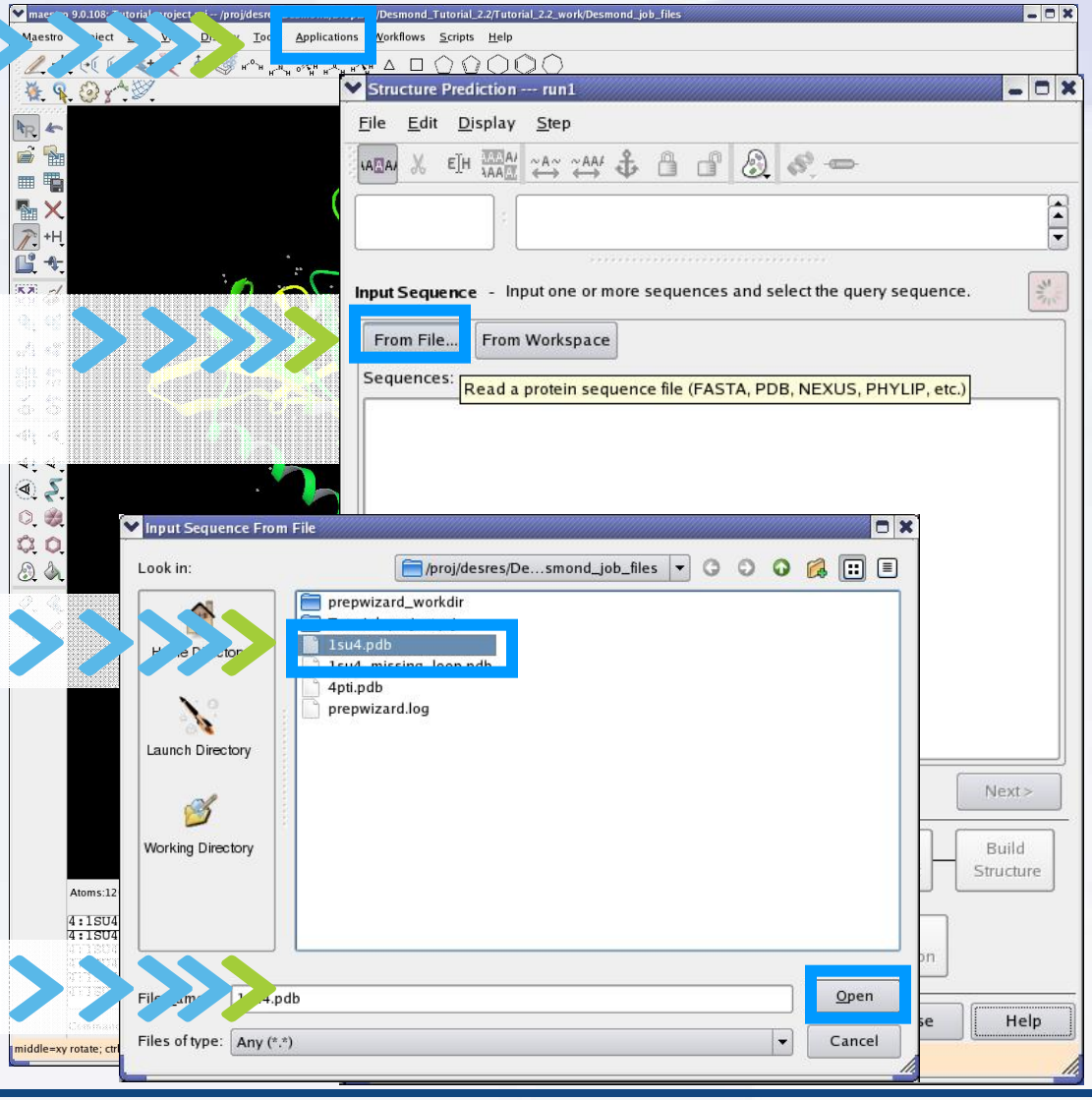
Running Prime

Select Applications>Prime> Structure Prediction

Click From File... to read in sequence

Select the original PDB file

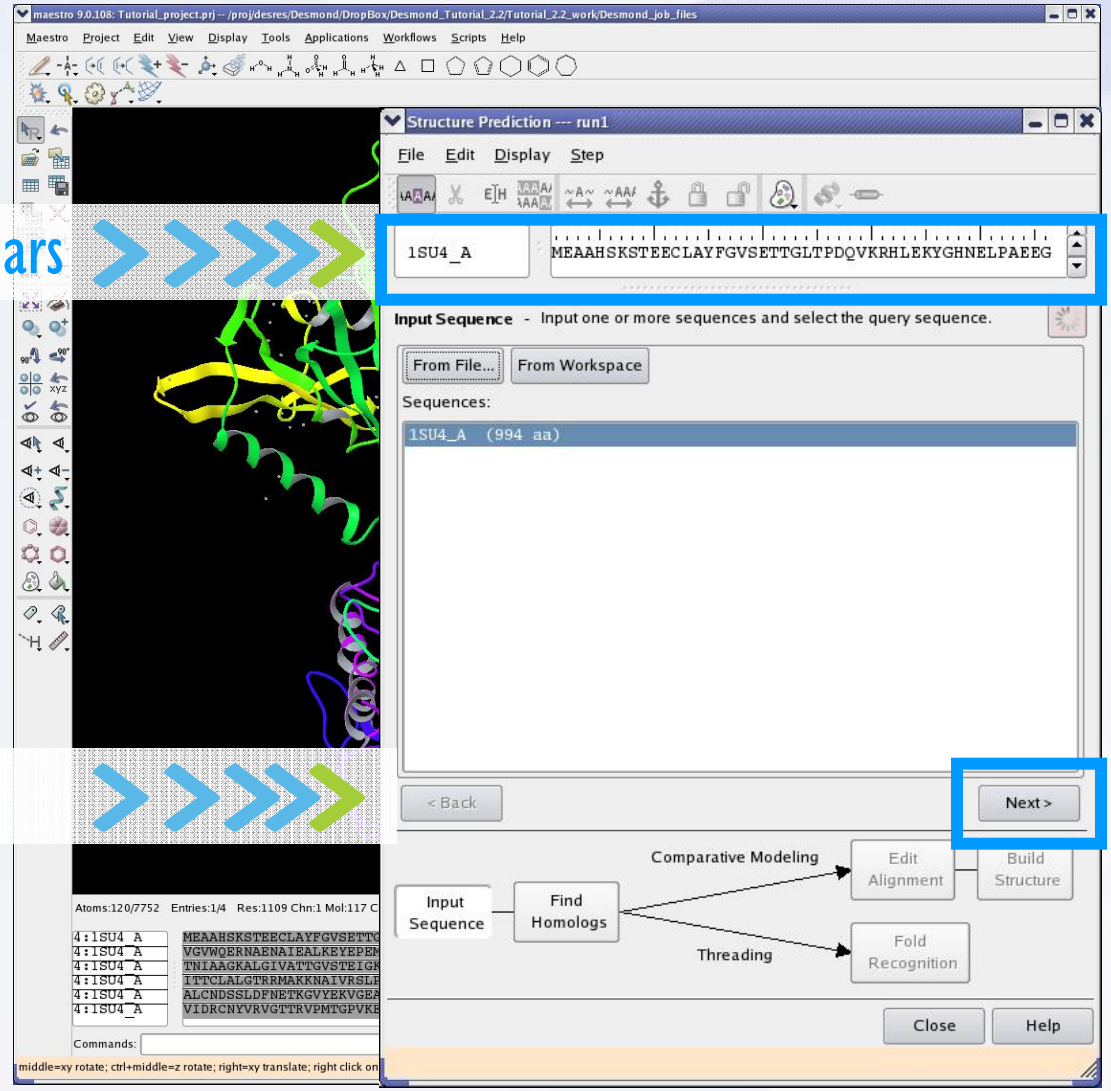
Click Open



Running Prime

Full sequence of residues appears

Click Next



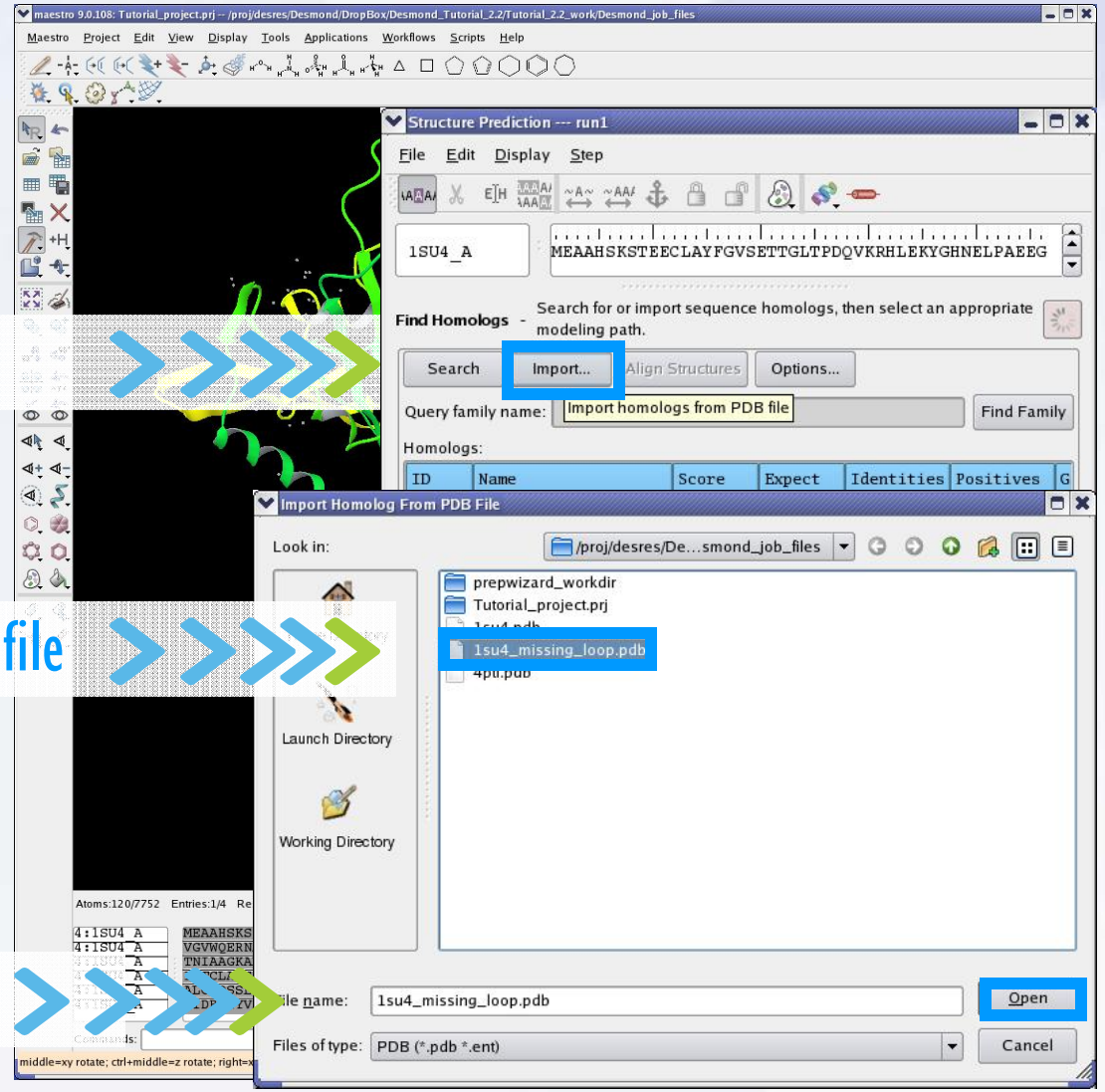
Running Prime

'Fool' Prime by using the truncated structure as a self-template

Click Import

Select the truncated structure file

Click Open

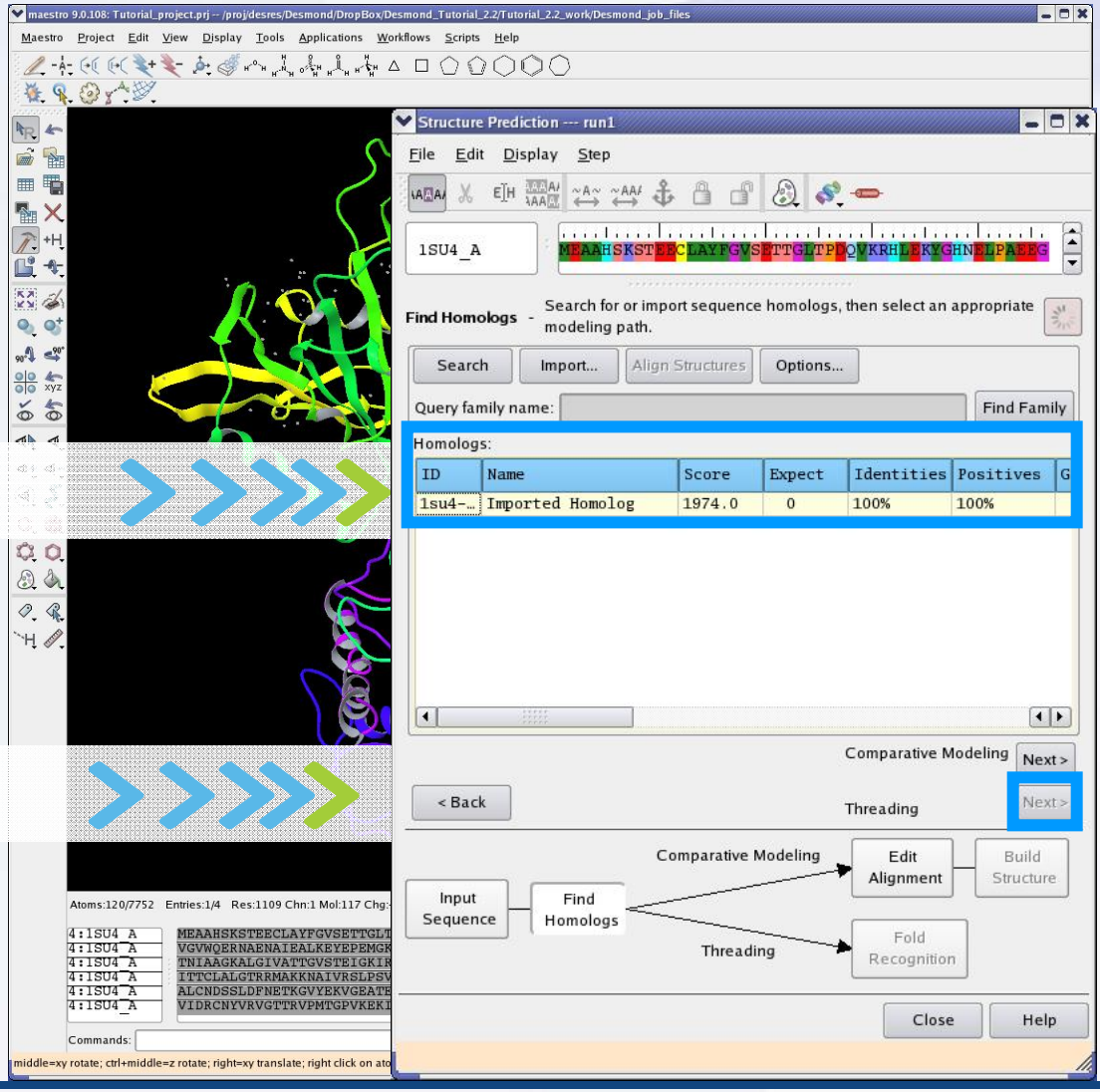


Running Prime

Click the ID column for the yellow line to see the sequence alignment at the top of the panel

Sequence identity is 100%

Click **Next** & click **Next** again

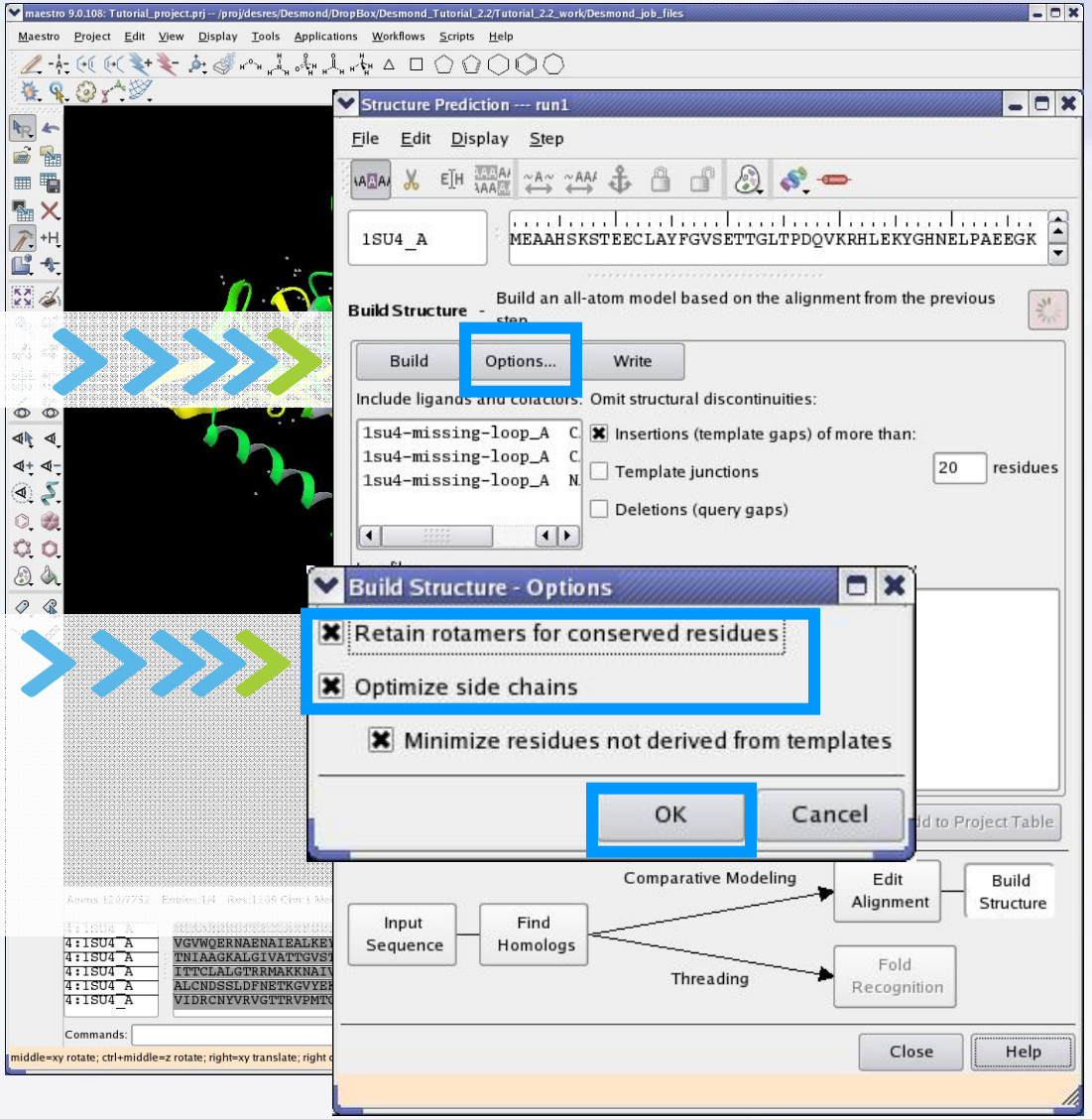


Running Prime

Click **Options**

Select the **Retain rotamers for conserved residues** and **Optimize side chains** options

Click **OK**



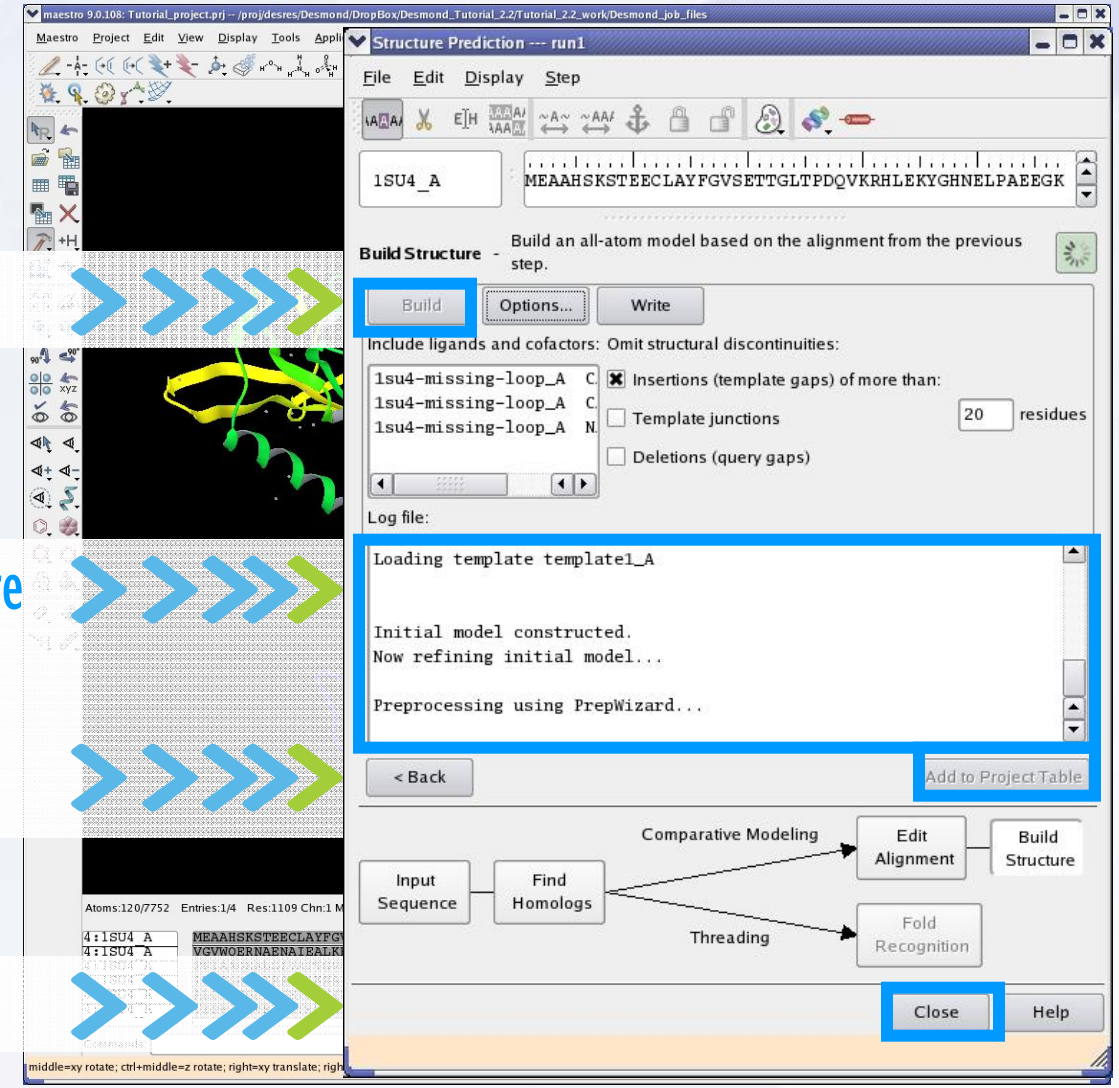
Running Prime

Click **Build**

Results appear as the structure is built

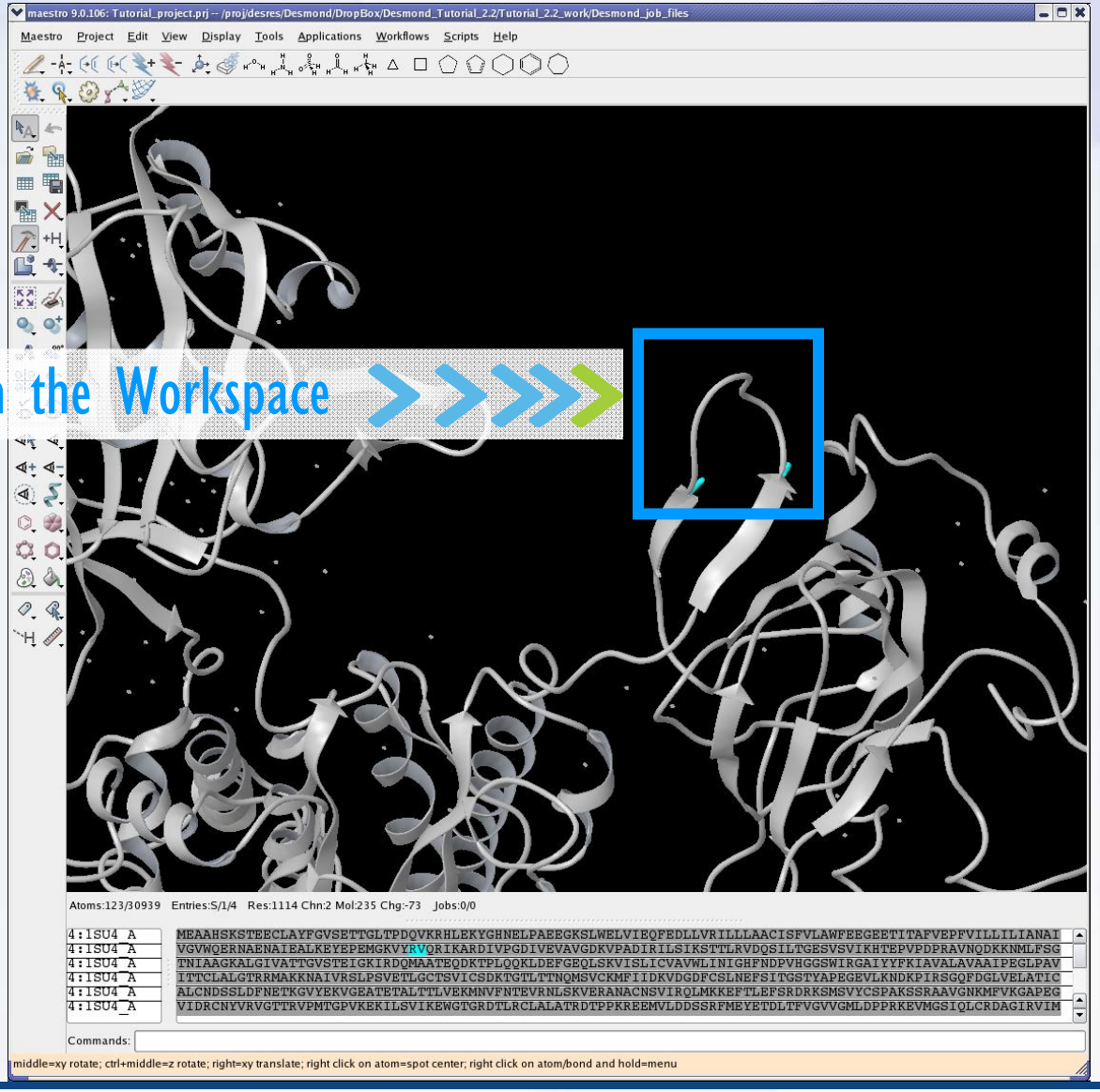
Click **Add to Project Table**

Click **Close**



Running Prime

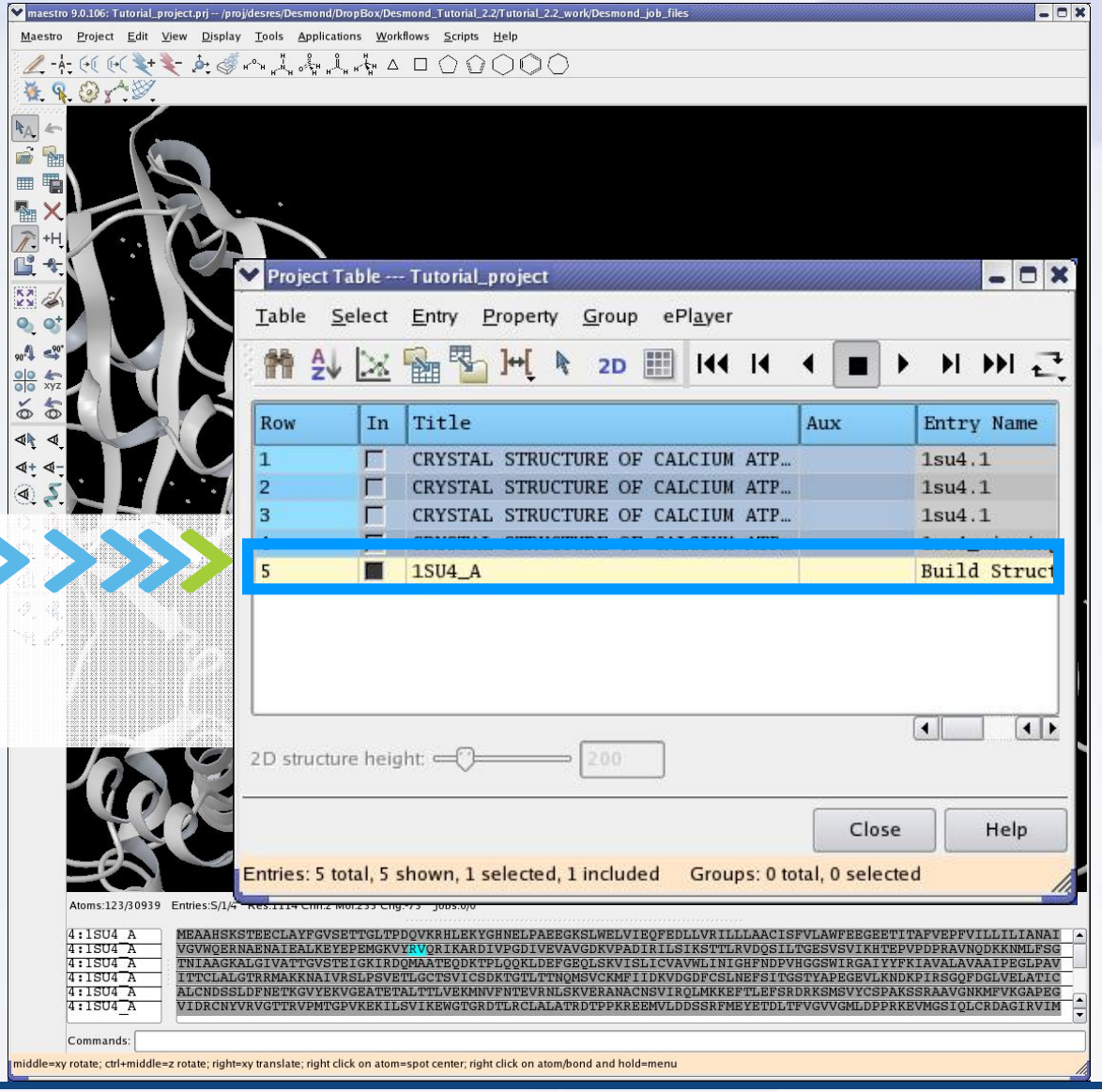
The built-in loop appears in the Workspace


maestro 9.0.106: Tutorial_project.prj -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:123/30939 Entries:S/1/4 Res:1114 Chn:2 Mol:235 Chg:-73 Jobs:0/0
4:1SU4_A
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Next refine the built-in loop with Prime Refinement

Running Prime

Make sure the last entry in the Project Table is included in the Workspace


maestro 9.0.106: Tutorial_project.prj -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Project Table --- Tutorial_project
Table Select Entry Property Group ePlayer
2D
Row In Title Aux Entry Name
1 CRYSTAL STRUCTURE OF CALCIUM ATP… 1su4.1
2 CRYSTAL STRUCTURE OF CALCIUM ATP… 1su4.1
3 CRYSTAL STRUCTURE OF CALCIUM ATP… 1su4.1
5 1SU4_A Build Struct
2D structure height: 200
Close Help
Entries: 5 total, 5 shown, 1 selected, 1 included Groups: 0 total, 0 selected
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

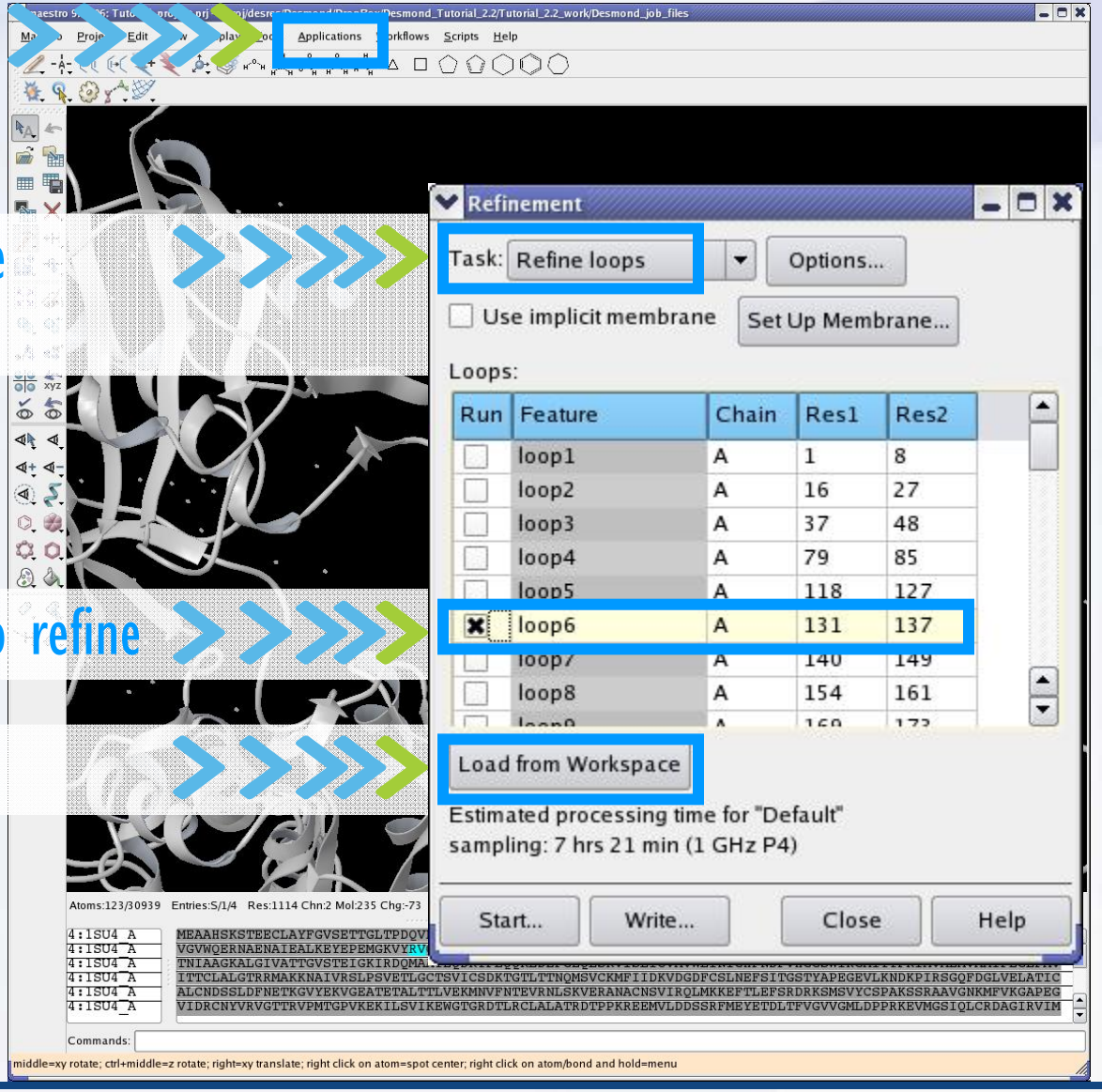
Running Prime

Select Applications>Prime>Refinement

Select Refine loops from the Task field

Select the loop you want to refine

Click Load from Workspace

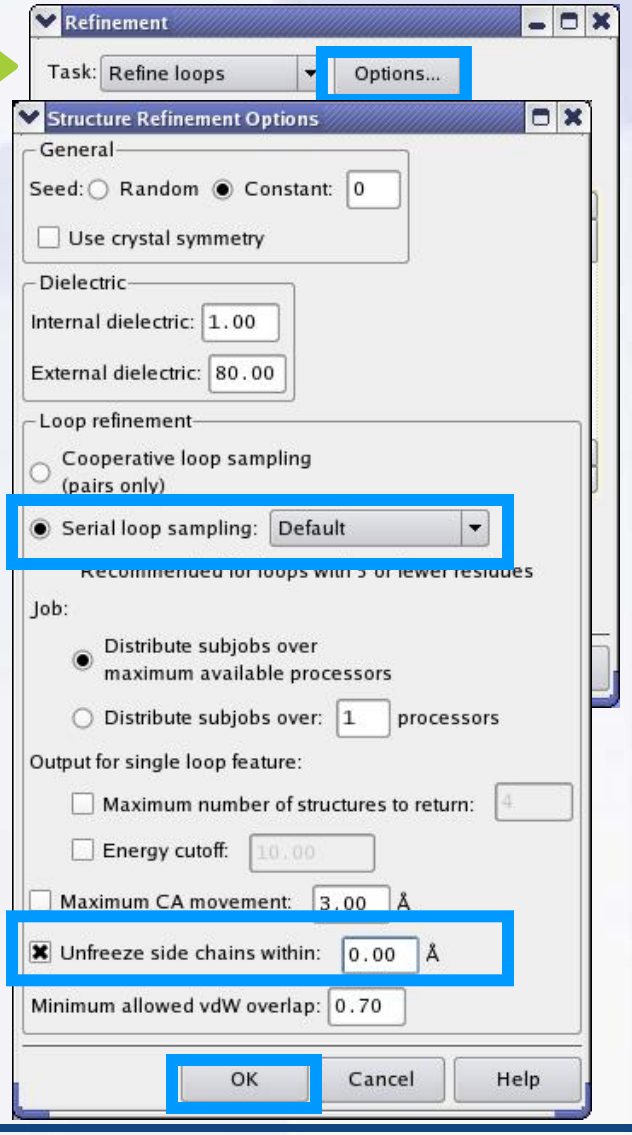


Running Prime

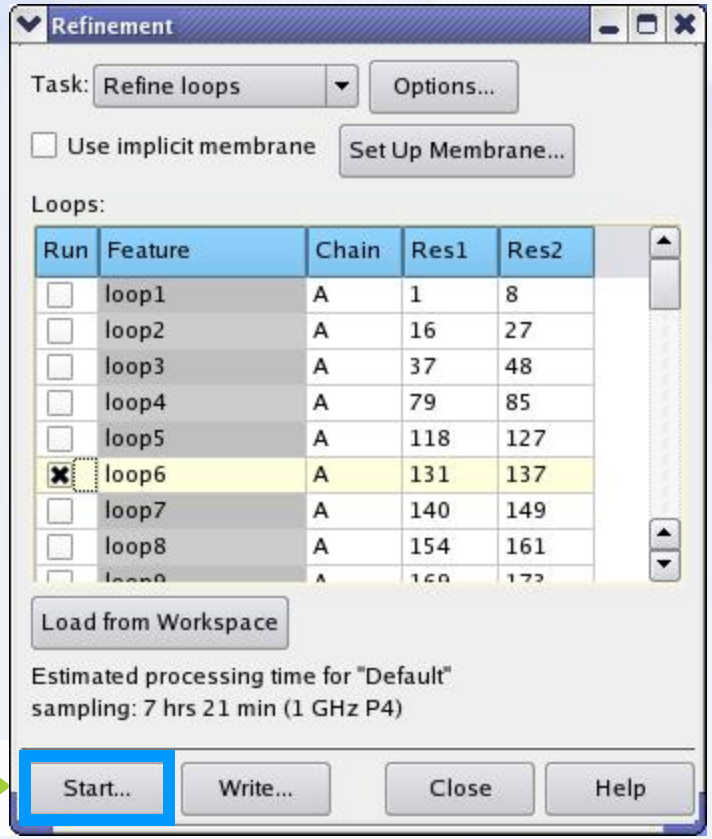
Click **Options**

Select the **Serial loop sampling** option

Select the **Unfreeze side chains within** option and enter 0.0 Å so side chains do not move



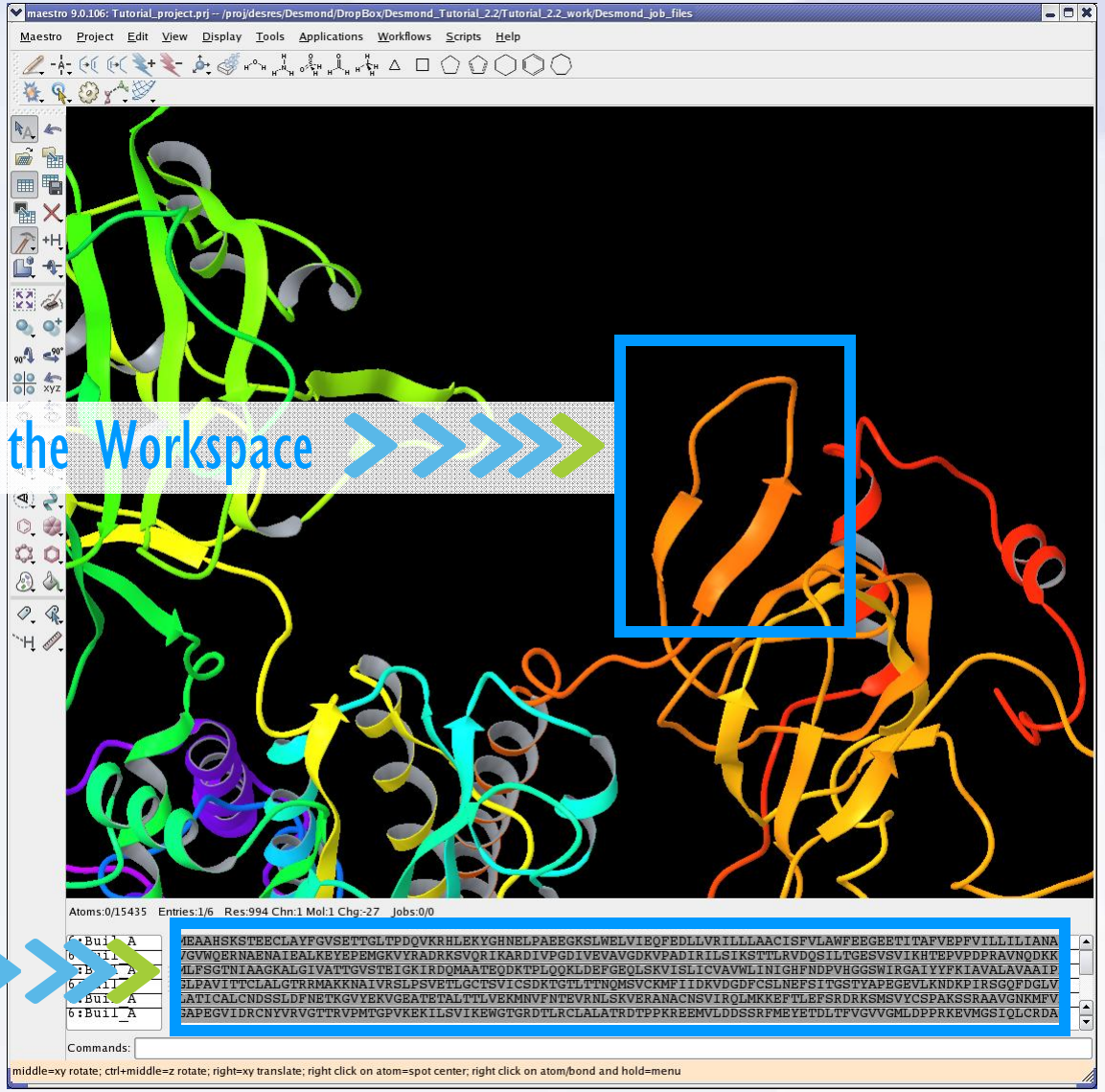
Running Prime



Click Start

Running Prime

Refinement should finish in less than 10 minutes


maestro 9.0.106: Tutorial_project.prj -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:0/15435 Entries:1/6 Res:994 Chn:1 Mol:1 Chg:-27 Jobs:0/0
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

The refined loop appears in the Workspace >>>>>

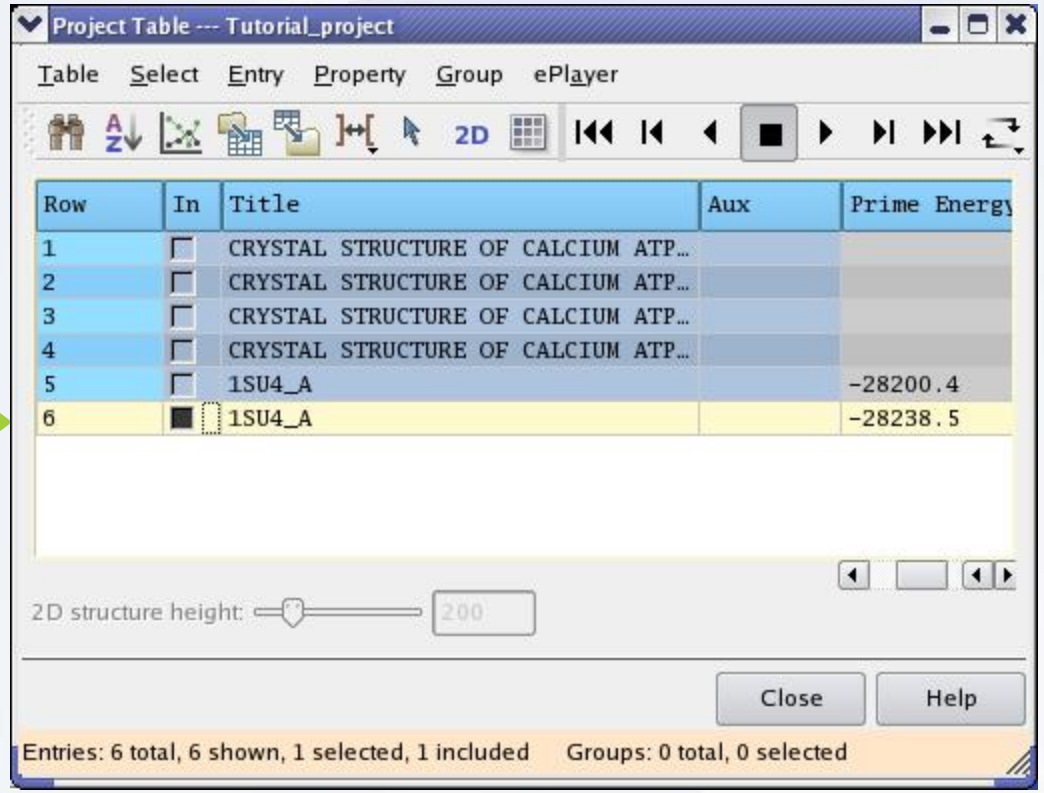
There is no longer a gap in the Sequence Viewer >>>>>

Running Prime

The Prime energy of the structure is lower with the refined loop than with the initial loop

The current state of the Project Table after loop refinement

- 1 (X-ray structure)
- 5 (initial loop)
- 6 (refined loop)


Project Table --- Tutorial_project
Table Select Entry Property Group ePlayer
2D
Row In Title Aux Prime Energy
1 CRYSTAL STRUCTURE OF CALCIUM ATP…
2 CRYSTAL STRUCTURE OF CALCIUM ATP…
3 CRYSTAL STRUCTURE OF CALCIUM ATP…
4 CRYSTAL STRUCTURE OF CALCIUM ATP…
5 1SU4_A -28200.4
6 1SU4_A -28238.5
2D structure height: 200
Close Help
Entries: 6 total, 6 shown, 1 selected, 1 included Groups: 0 total, 0 selected

Running Prime

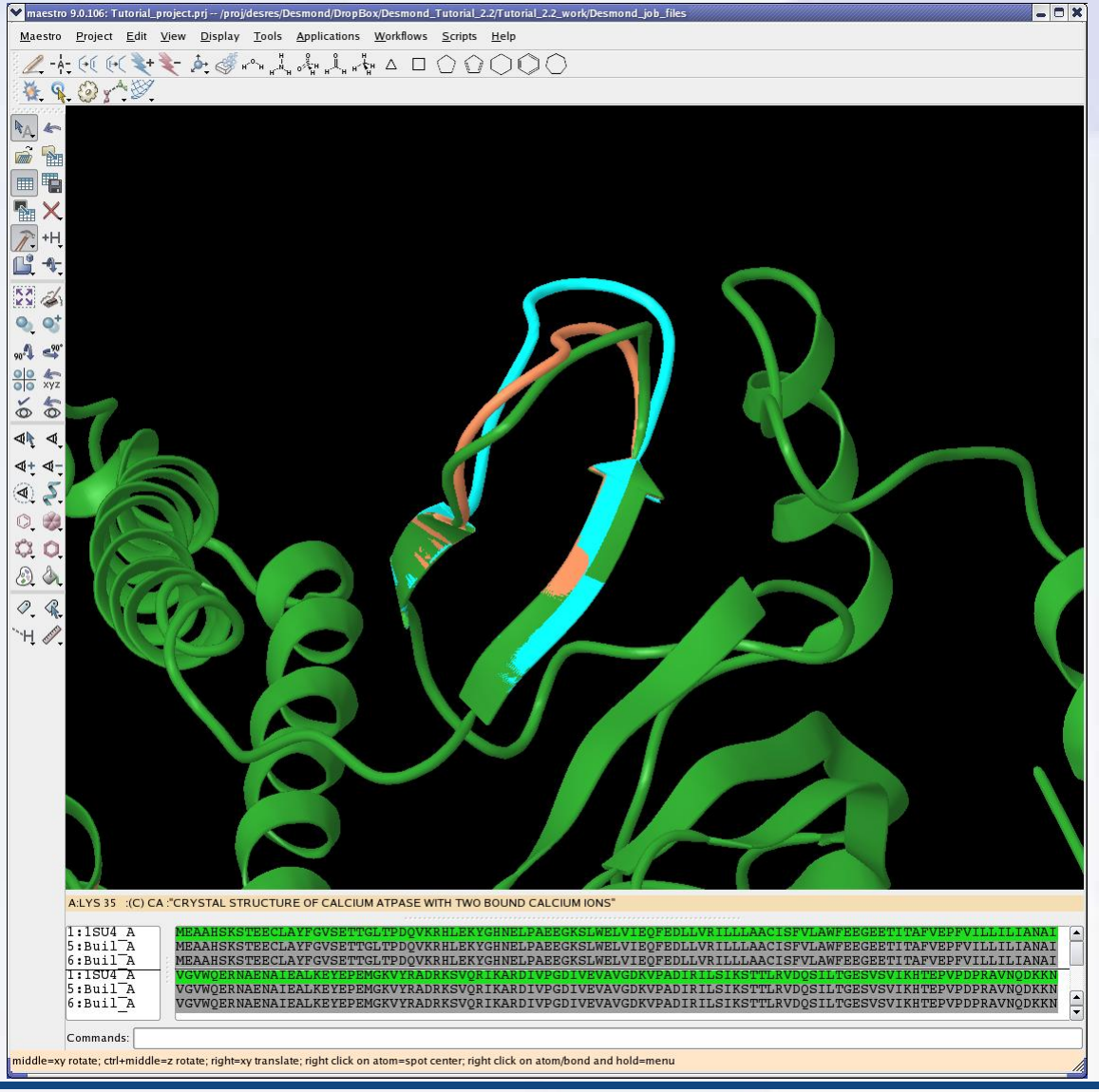
Superposition of 3 loops:

- Cyan loop is original
- Brown loop is initial build
- Green loop is refined

Show entries from the Project Table in the Workspace

Select **Display>Ribbons**

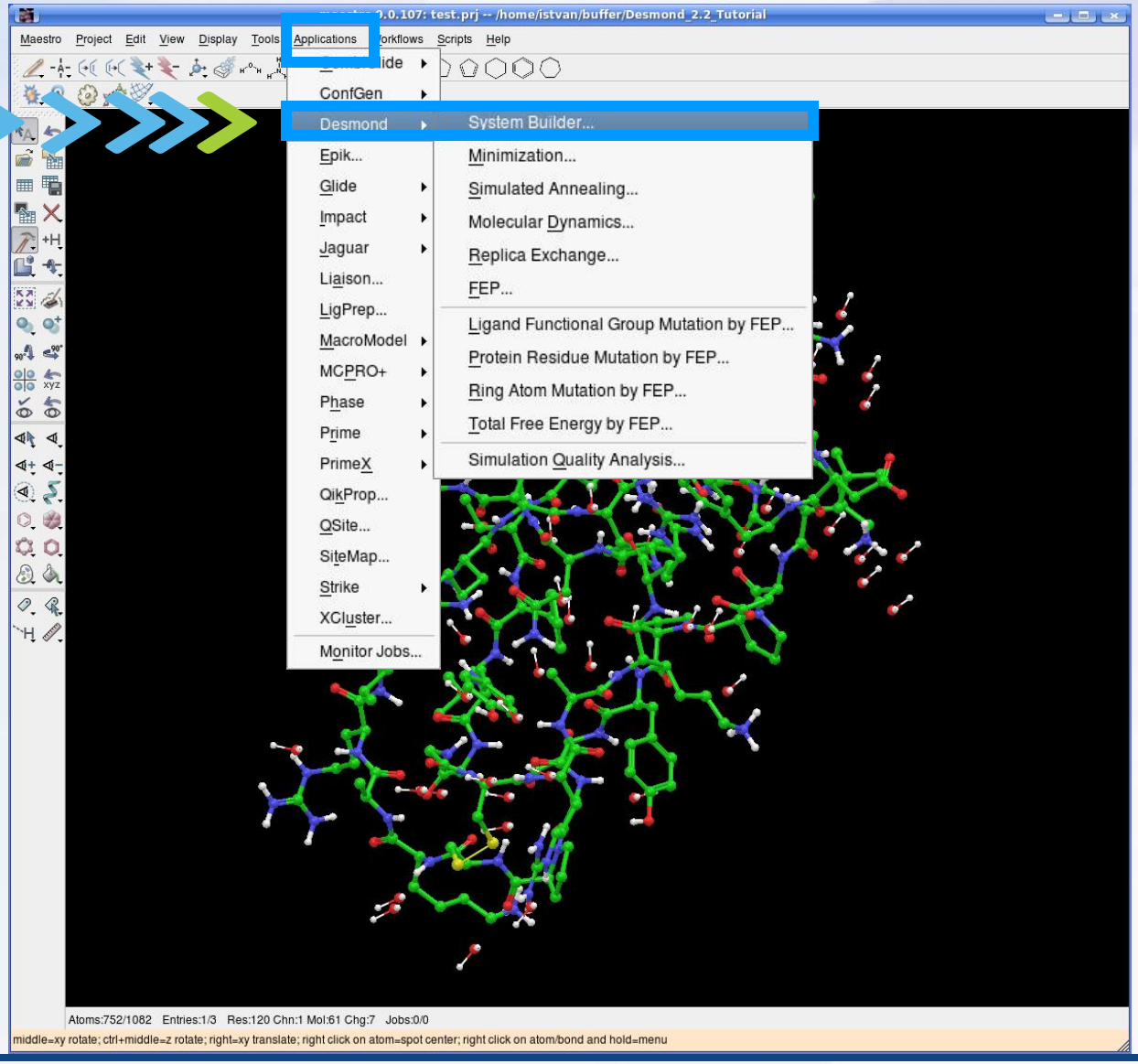
Recolor with '**Entry**' scheme


maestro 9.0.106: Tutorial_project.prj -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
A:LYS 35 :(C) CA :"CRYSTAL STRUCTURE OF CALCIUM ATPASE WITH TWO BOUND CALCIUM IONS"
1:lSU4_A MEAAHSKSTEECLAYFGVSETTGLTPDQVKRHLEKYGHNELPAEEGKSLWELVIEQFEDLLVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILIANAI
5:Buil_A MEAAHSKSTEECLAYFGVSETTGLTPDQVKRHLEKYGHNELPAEEGKSLWELVIEQFEDLLVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILIANAI
6:Buil_A MEAAHSKSTEECLAYFGVSETTGLTPDQVKRHLEKYGHNELPAEEGKSLWELVIEQFEDLLVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILIANAI
1:lSU4_A VGVWQERNAENAIEALKEYEPEMGKVYRADRKSVQRIKARDIVPGDIVEVAVGDKVPADIRILSIKSTTLRVDQSILTGESVSVIKHTEPVPDPRAVNQDKKN
5:Buil_A VGVWQERNAENAIEALKEYEPEMGKVYRADRKSVQRIKARDIVPGDIVEVAVGDKVPADIRILSIKSTTLRVDQSILTGESVSVIKHTEPVPDPRAVNQDKKN
6:Buil_A VGVWQERNAENAIEALKEYEPEMGKVYRADRKSVQRIKARDIVPGDIVEVAVGDKVPADIRILSIKSTTLRVDQSILTGESVSVIKHTEPVPDPRAVNQDKKN
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Preparing a Desmond simulation

Select Applications> Desmond>System Builder

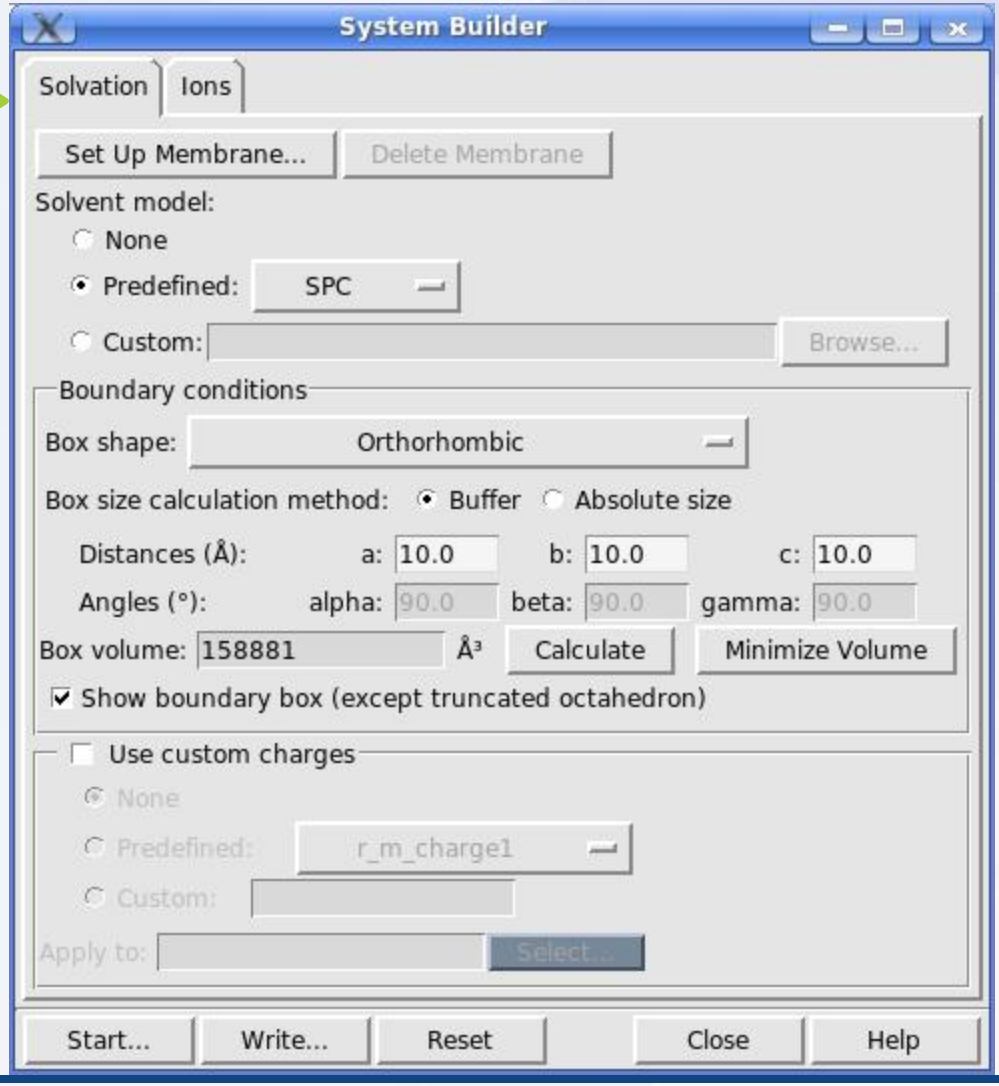
System Builder lets you generate a solvated system for Desmond simulations



Preparing a Desmond simulation

System Builder opens

System Builder generates a solvated system that includes the solute, solvent, and counter and salt ions



Selecting solutes and solvents

- The current contents of the Workspace constitute the solute (visible or not visible)
- Supported solvent models include SPC, TIP3P, TIP4P and TIP4PEW water, and organic solvents DMSO, methanol and octanol
- Viparr allows TIP5P and other water models
- Custom models are allowed if you provide a pre-equilibrated simulation box of a different solvent molecule

Defining the simulation box

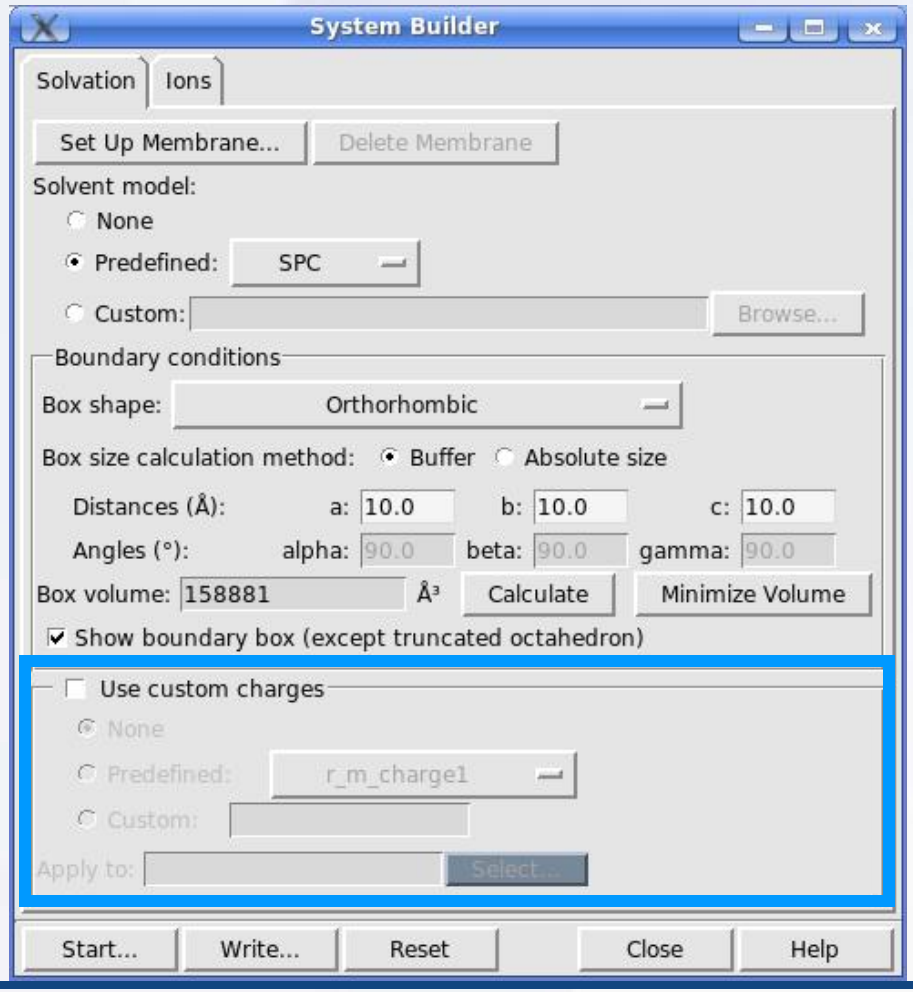
- **Goal:** Reduce the volume of solvent while ensuring that enough solvent surrounds the solute so that the protein does not 'see' a periodic image of itself during simulation
- Minimize solvent volume by:
  - Selecting shape that is similar to the protein structure
  - Applying a reasonable buffer (~10 Å, the typical Coulombic interaction cutoff for long-range electrostatic calculations)
  - Clicking the **Minimize Volume** button in System Builder
  - Applying the truncated octahedron box shape

System Builder output format

- Composite model system file with extension **.cms**
- Total solvated system decomposed into 5 sections:
  - Protein (solute)
  - Counter ions
  - Positive salt ions
  - Negative salt ions
  - Water molecules
- OPLS-AA force field parameters are also written to CMS file

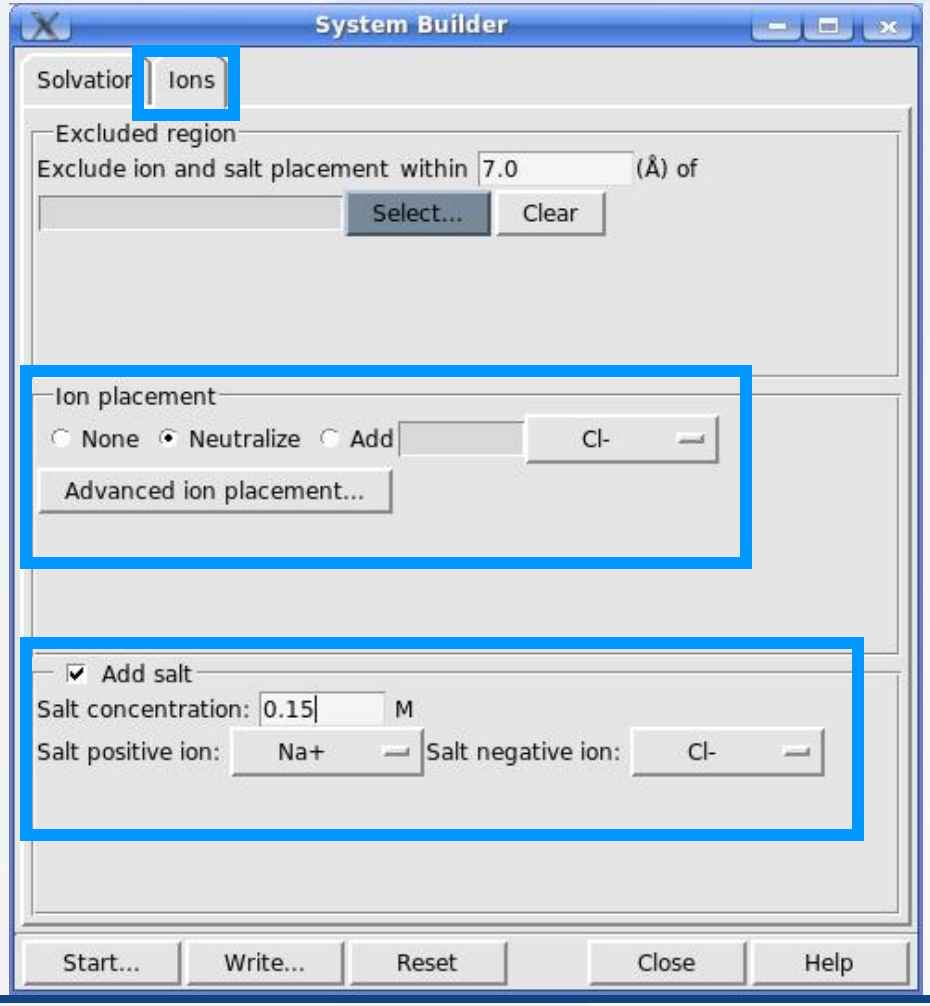
Adding custom charges

- Specify custom charges in the **Use custom charges** section of System Builder
  - Identify the charge to use
  - Specify subset of atoms for which custom charges will be applied using **Select** panel



Adding ions

- Add ions using the **Ions** tab of System Builder
  (Solute is neutralized by default)
  - Add an arbitrary number of counterions if there is a reason not to neutralize
  - Add background salt by setting the salt concentration in the **Add Salt** section
  - Specify the type of positive or negative ions to use



Generating the solvated system from command line

- Click the **Write** button in System Builder to save job files
  - **my_setup.mae** is the Maestro structure file of the solute
    Serves as the input for system setup
  - **my_setup.csb** is the command file
    Can be hand-edited for custom setup cases
  - **my_setup-out.cms** is the Maestro structure file of simulation system
- Execute this command at the command line:

```
$ $SCHRODINGER/run
$SCHRODINGER/utilities/system_builder my_setup.csb
```

where *my_setup* is the file name given to the Write operation

Setting up membrane systems

- Embed a membrane protein structure in a membrane bilayer using the **Setup Membrane** panel
  - An equilibrated membrane (including accompanying water) is used to generate a large enough region to encompass the protein
  - Protein is positioned within the membrane using a semi-automated procedure
- Example uses the calcium ATPase protein, 1su4

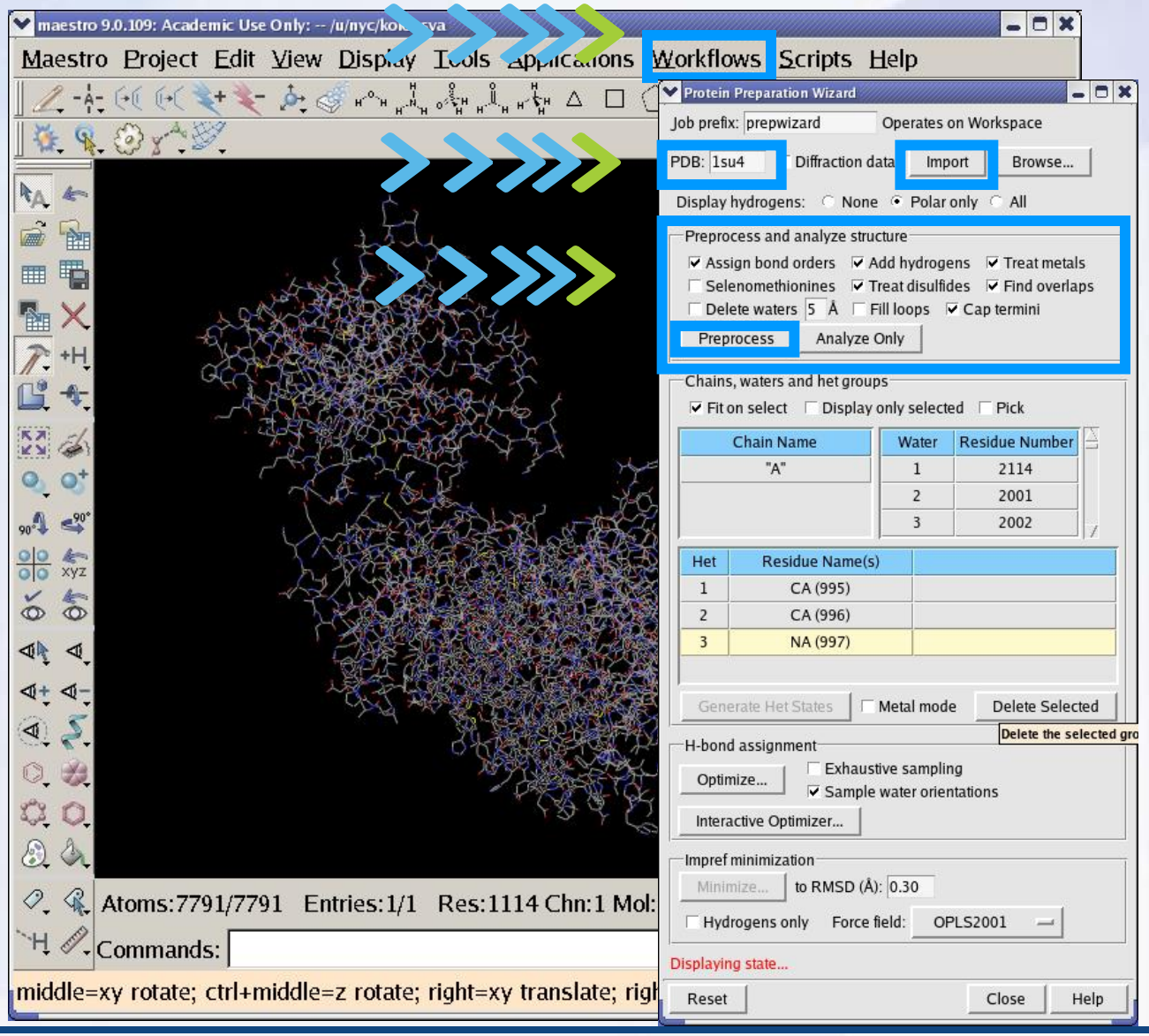
Setting up membrane systems

Select **Workflows > Protein Preparation Wizard**

Enter "1su4" in the **PDB** field and click **Import**

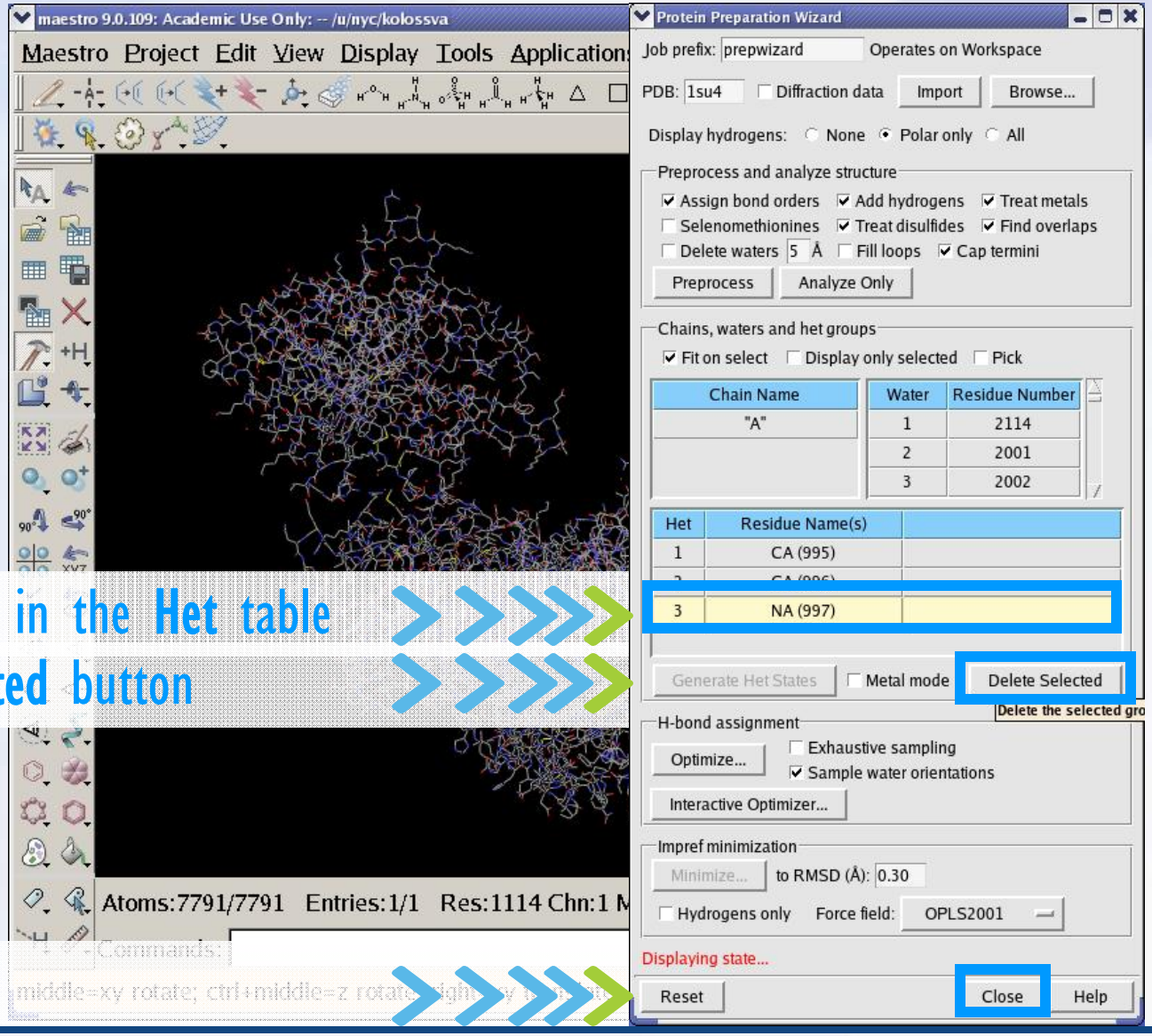
Check options for preprocessing as shown (do not delete crystal waters)

Click **Preprocess**



Setting up membrane systems

Sodium ion is just an artifact of the crystallization process and should be deleted



Select the sodium ion in the **Het** table

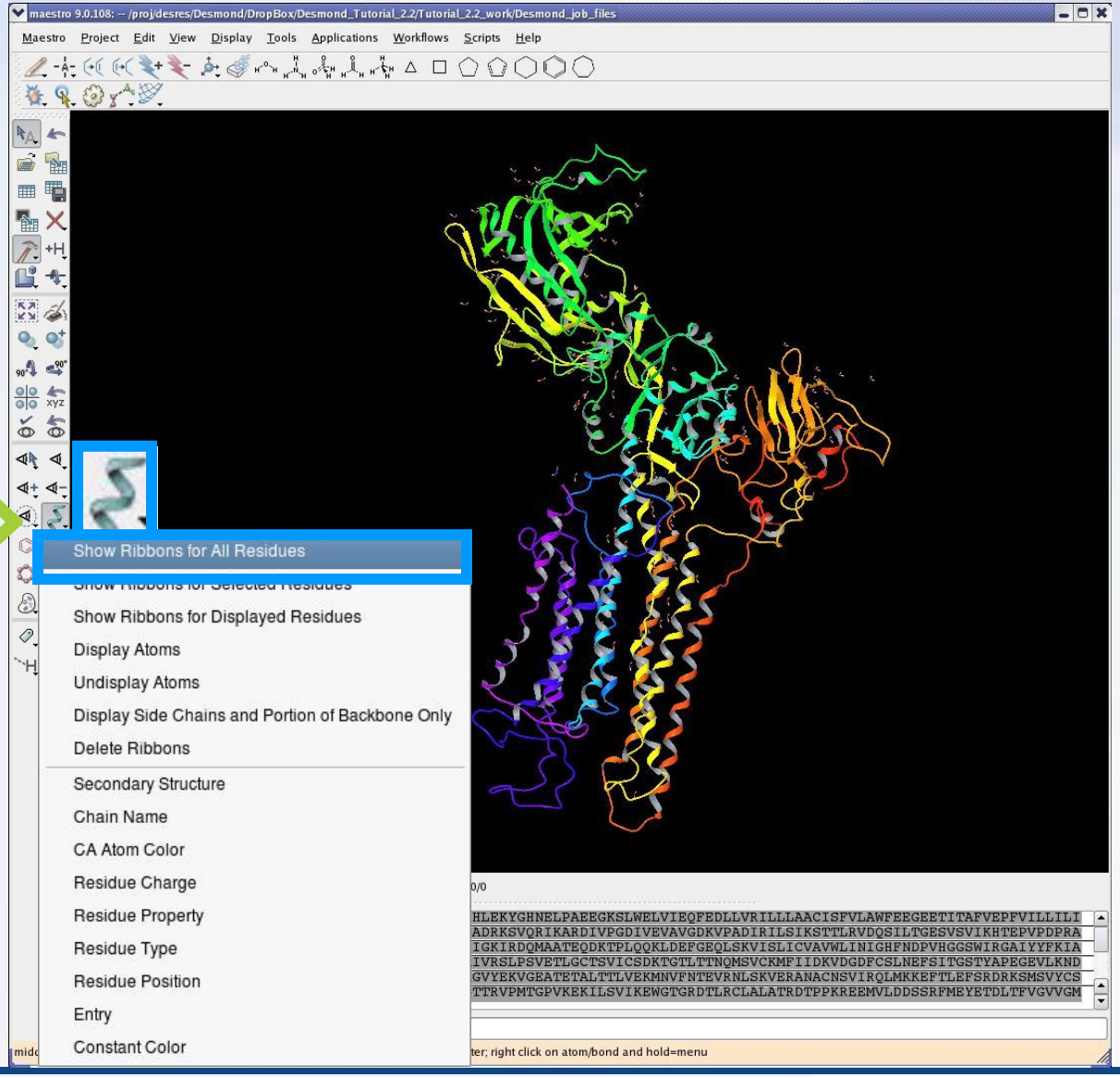
Click the **Delete Selected** button

Click **Close**

Setting up membrane systems

Click the **Ribbon** button and select **Show Ribbons for All Residues**

Orient the protein with the transmembrane bundle aligned along the vertical axis as shown

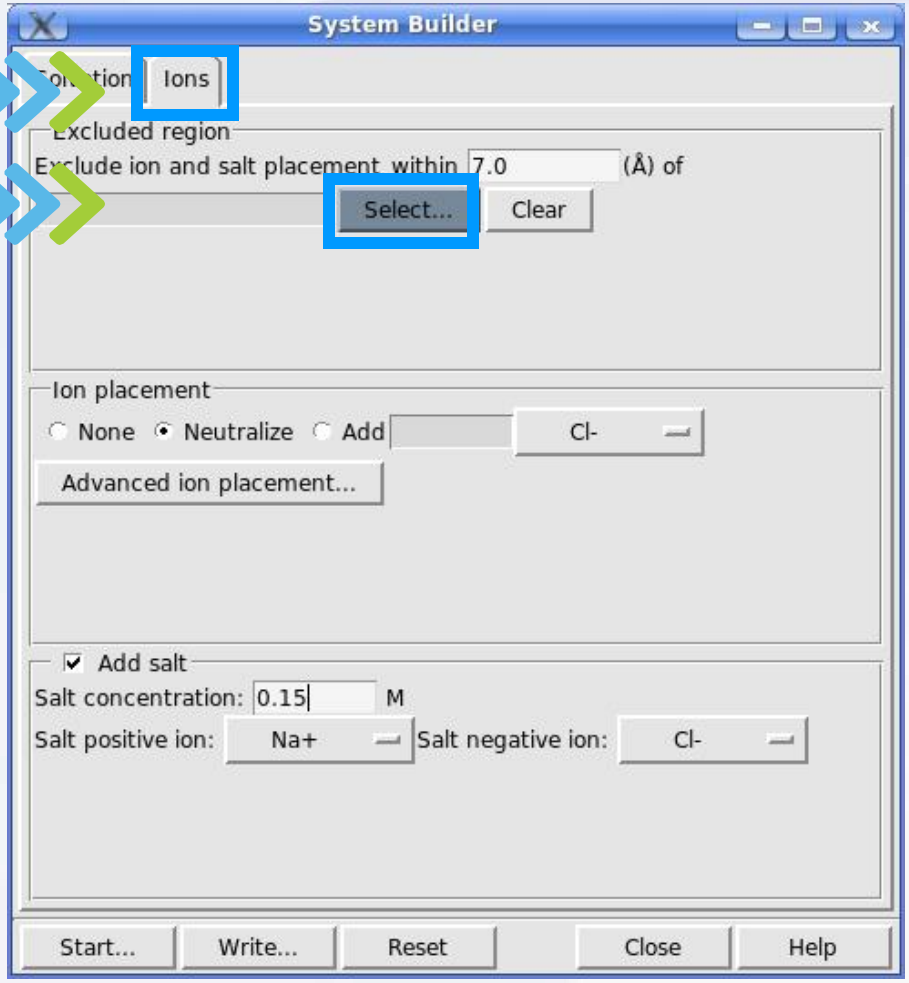


Setting up membrane systems

Select Applications>Desmond >System Builder

Click the Ions tab

Click the Select button



Setting up membrane systems

Click the **Molecule** tab

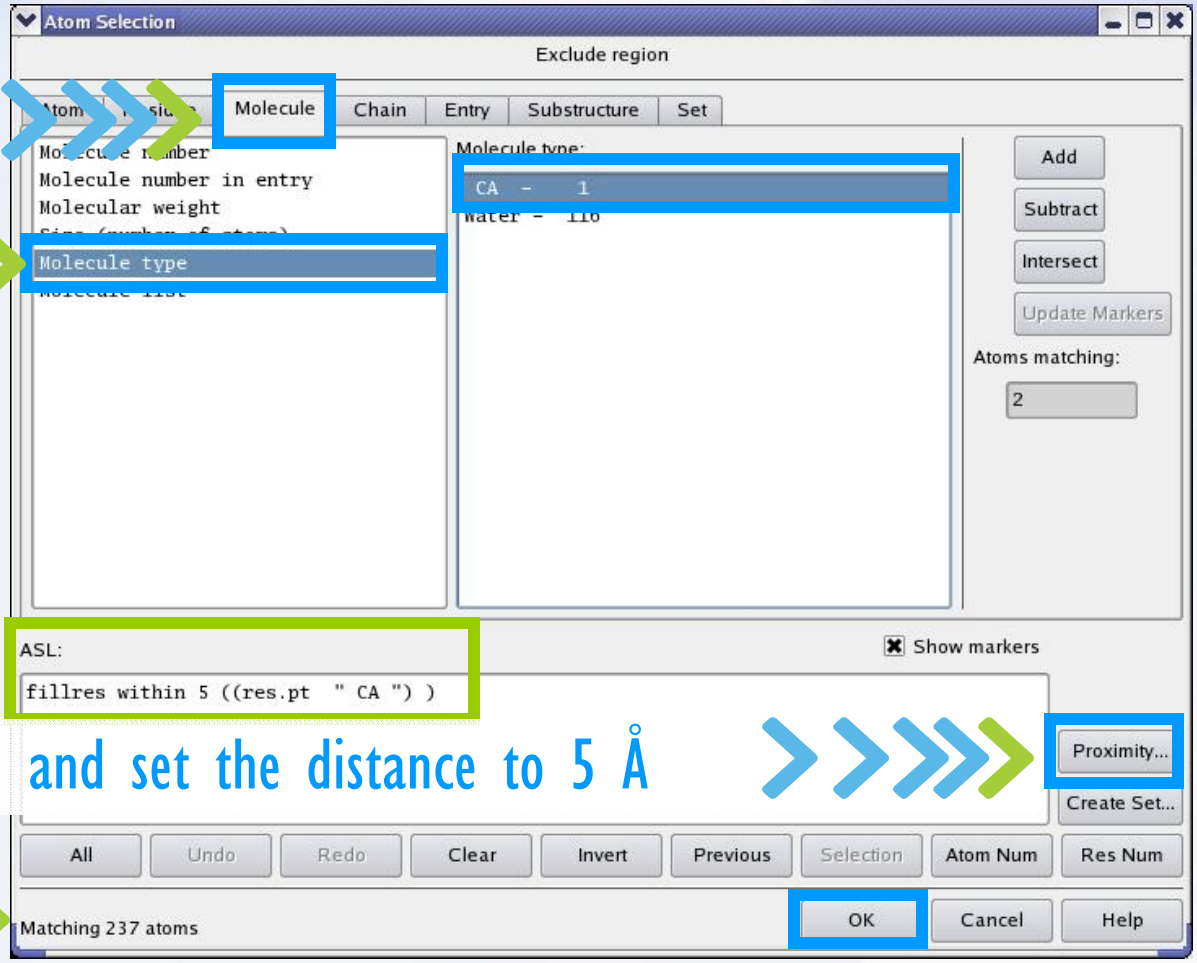
Select **Molecule type**

Select the **CA** ions

The **ASL** area defines the excluded region

Click the **Proximity** button and set the distance to 5 Å

Click **OK**



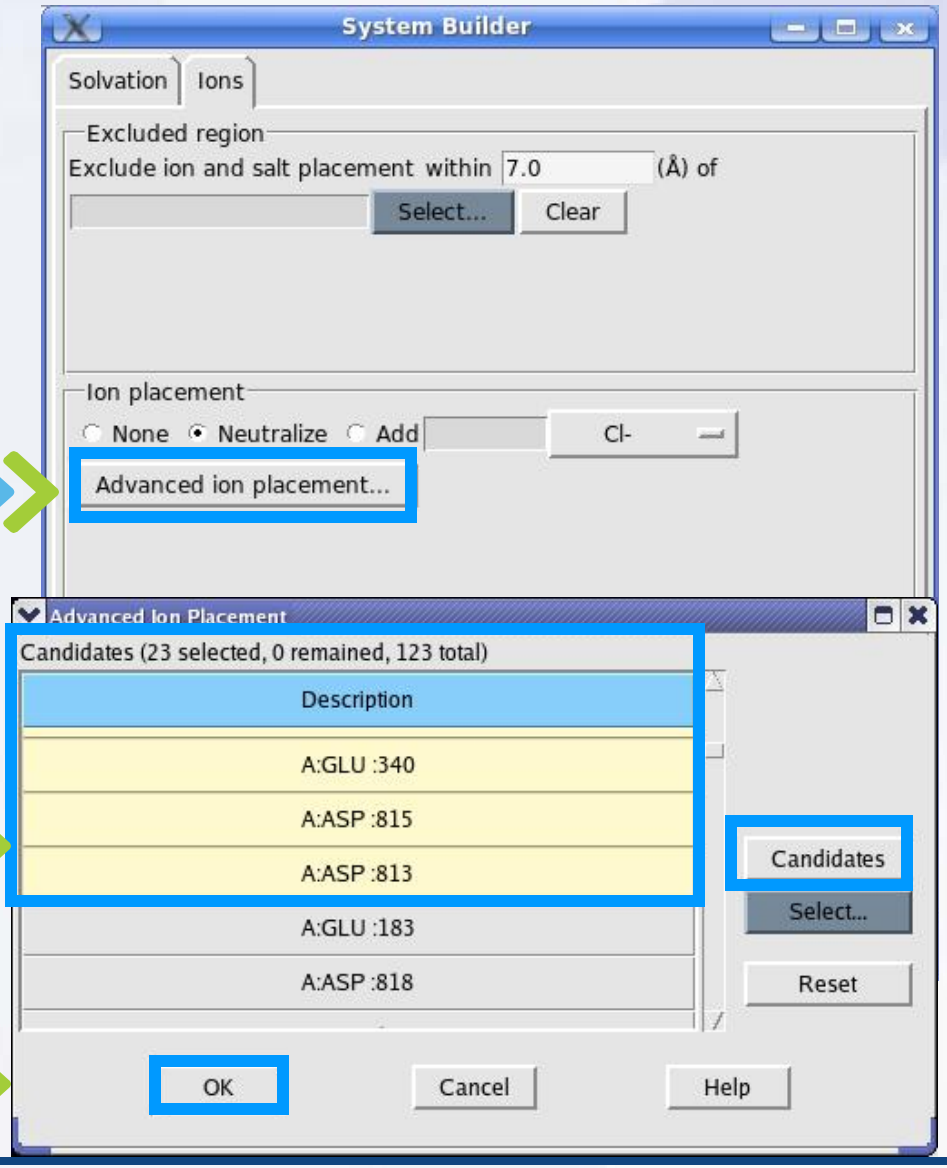
Setting up membrane systems

Click the **Advanced ion placement** button

Click the **Candidates** button

Shift-click to select the first 23 residues under **Candidates**

Click **OK**

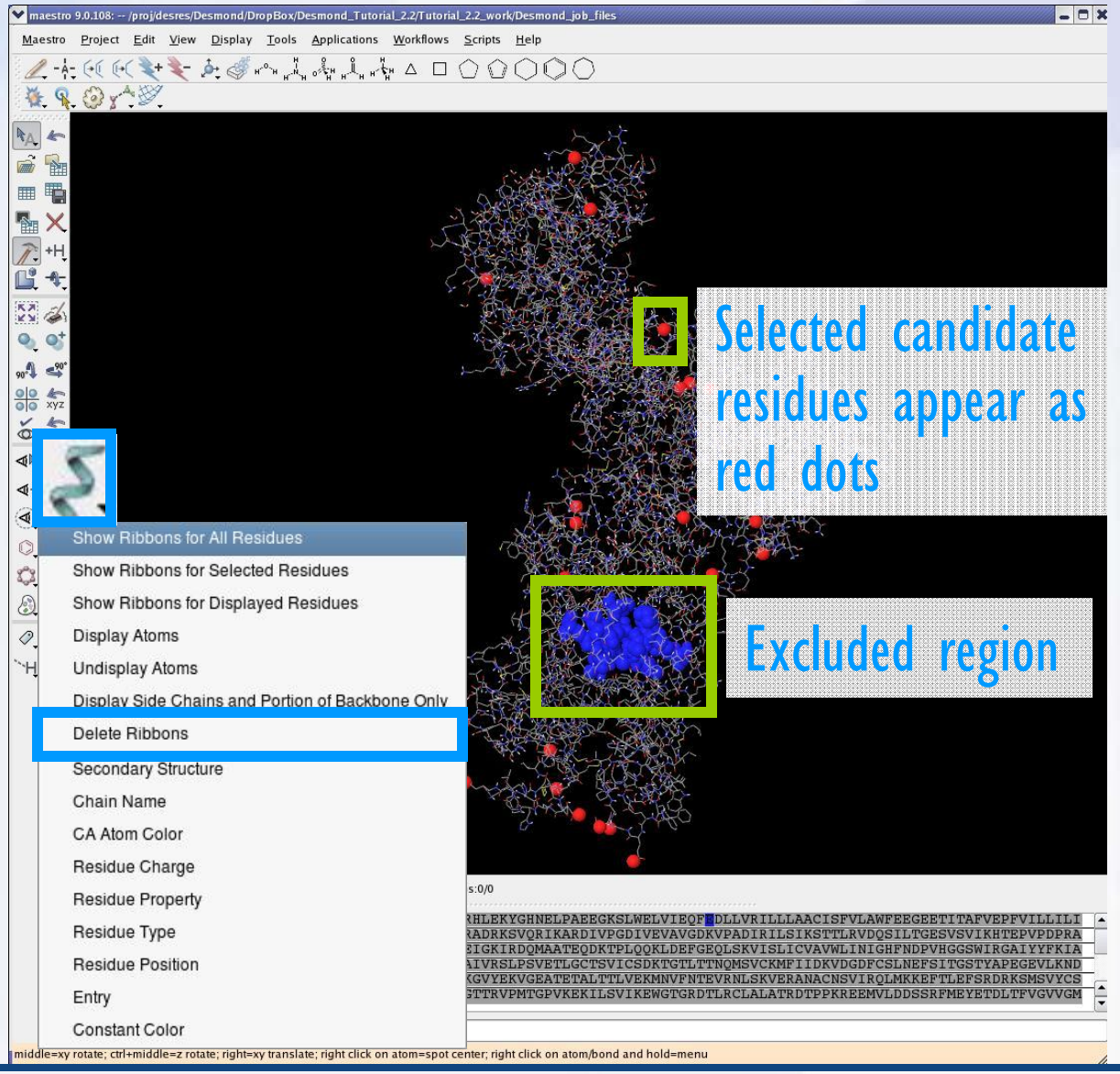


Setting up membrane systems

Turn off ribbon view to see candidate selection in the Workspace

Click the Ribbon button…

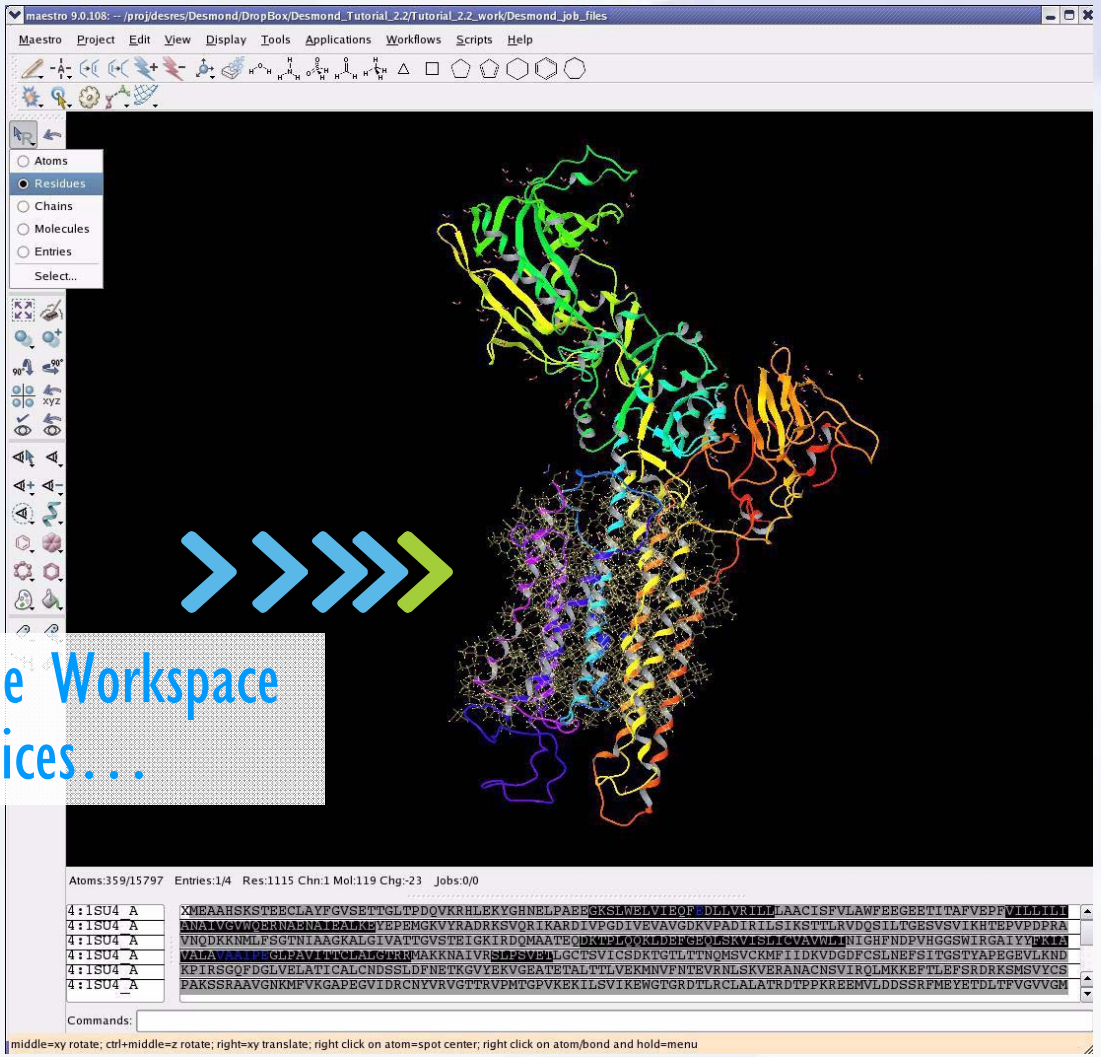
Select Delete Ribbons


maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Selected candidate residues appear as red dots
Excluded region
Show Ribbons for All Residues
Show Ribbons for Selected Residues
Show Ribbons for Displayed Residues
Display Atoms
Undisplay Atoms
Display Side Chains and Portion of Backbone Only
Delete Ribbons
Secondary Structure
Chain Name
CA Atom Color
Residue Charge
Residue Property
Residue Type
Residue Position
Entry
Constant Color
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Setting up membrane systems

Change the toolbar **Select** icon to **R** for residue selection…

Select a rectangular area in the Workspace around the transmembrane helices…

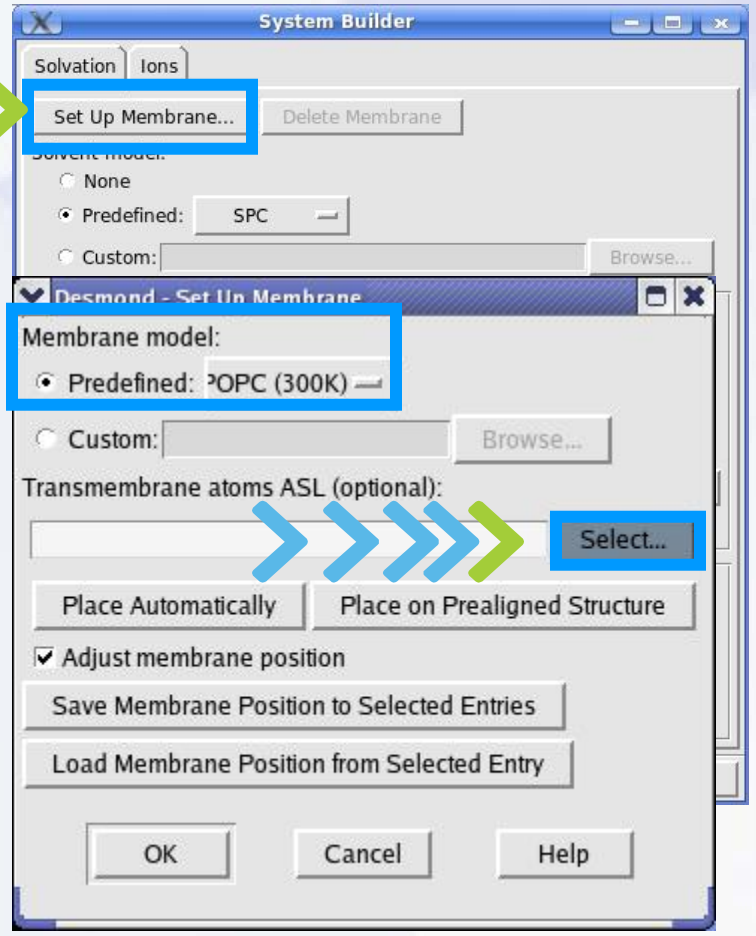


Setting up membrane systems

Click the **Set Up Membrane** button

Select **POPC** as the membrane model

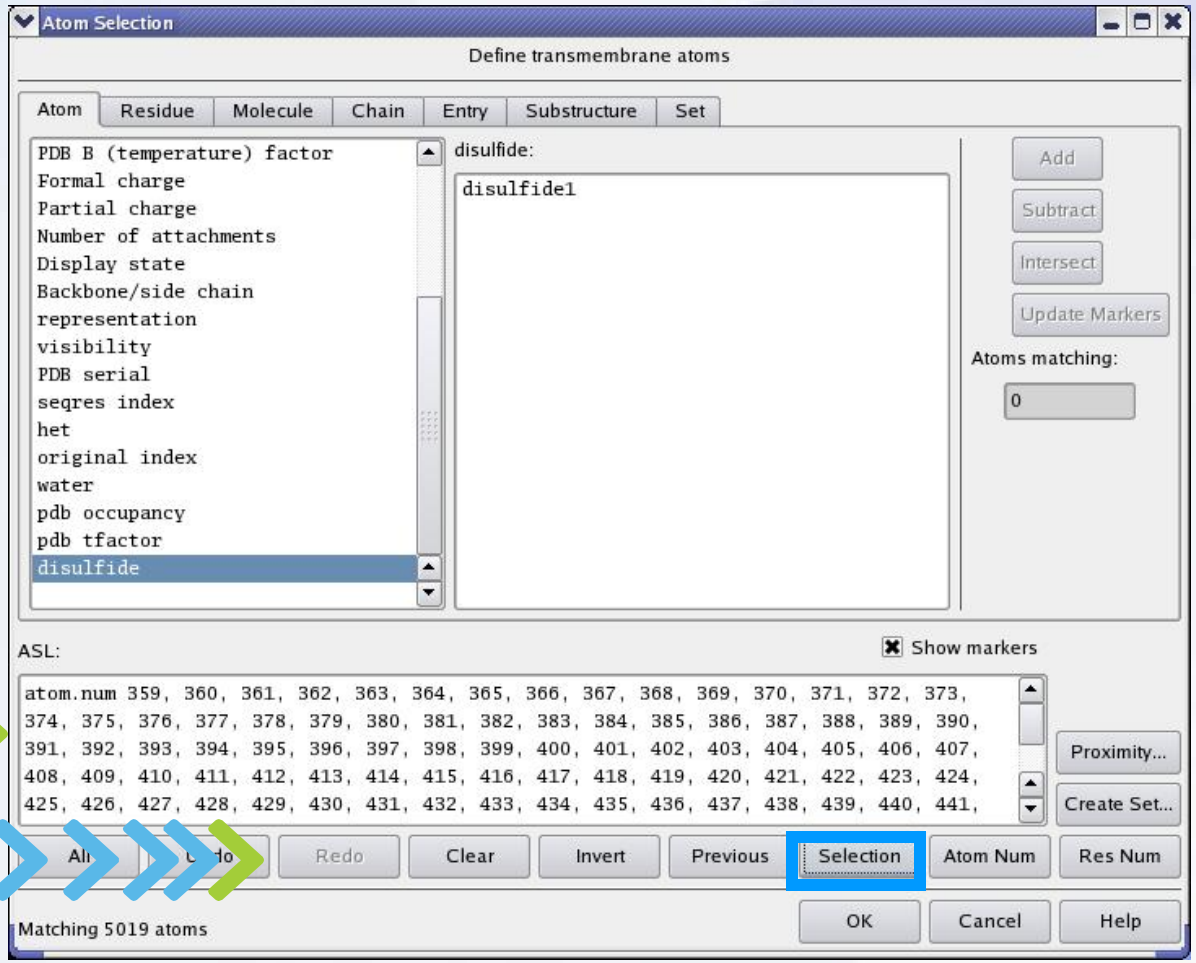
Click the **Select** button



Setting up membrane systems

Wait until the ASL area is filled

Click the Selection button

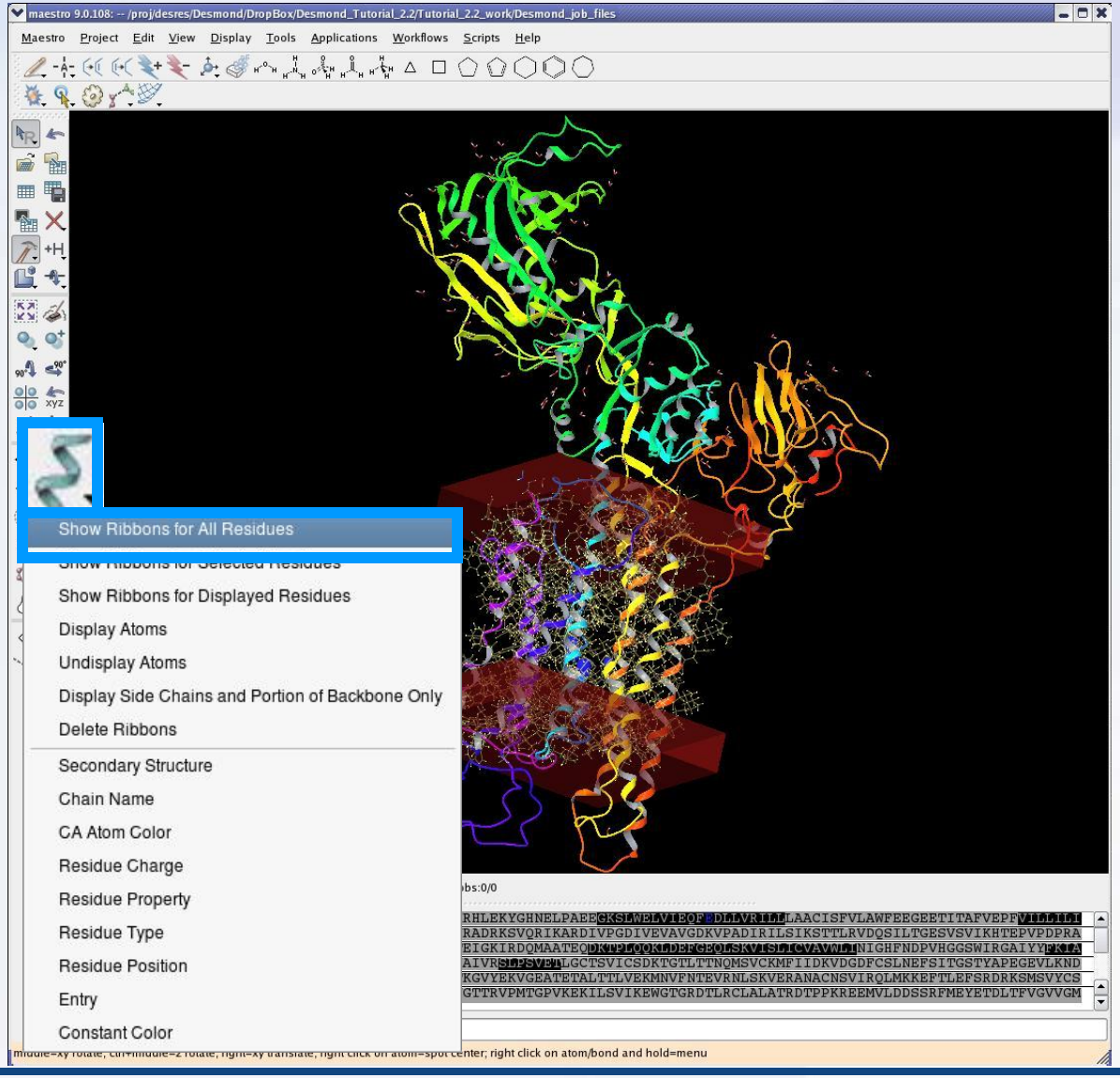


Setting up membrane systems

Switch to ribbon view

Workspace shows the initial automatic placement for the protein

Click the >>>>> Ribbon button and select Show Ribbons for All Residues

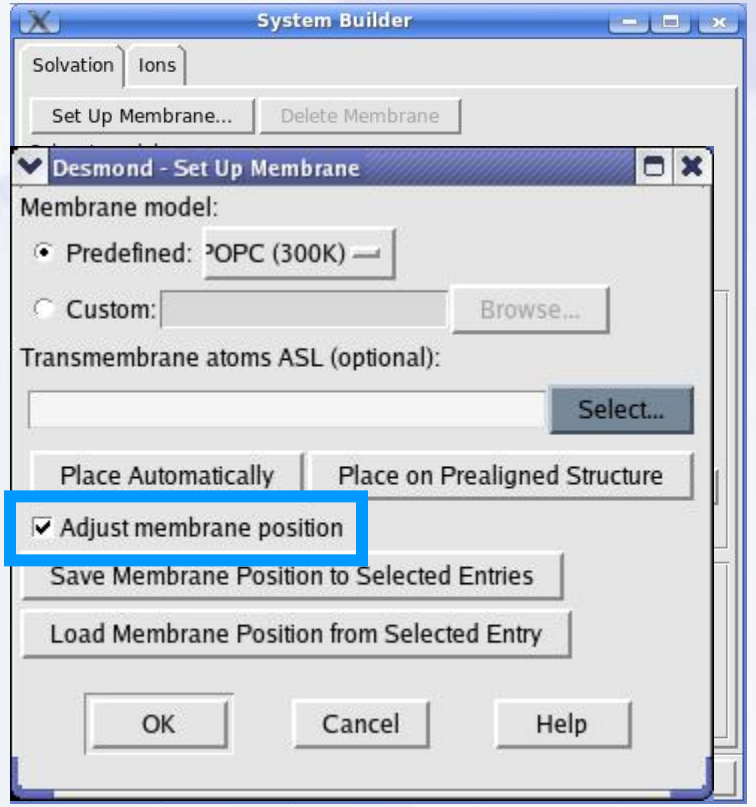


Setting up membrane systems

Adjust the *local scope* for transformations

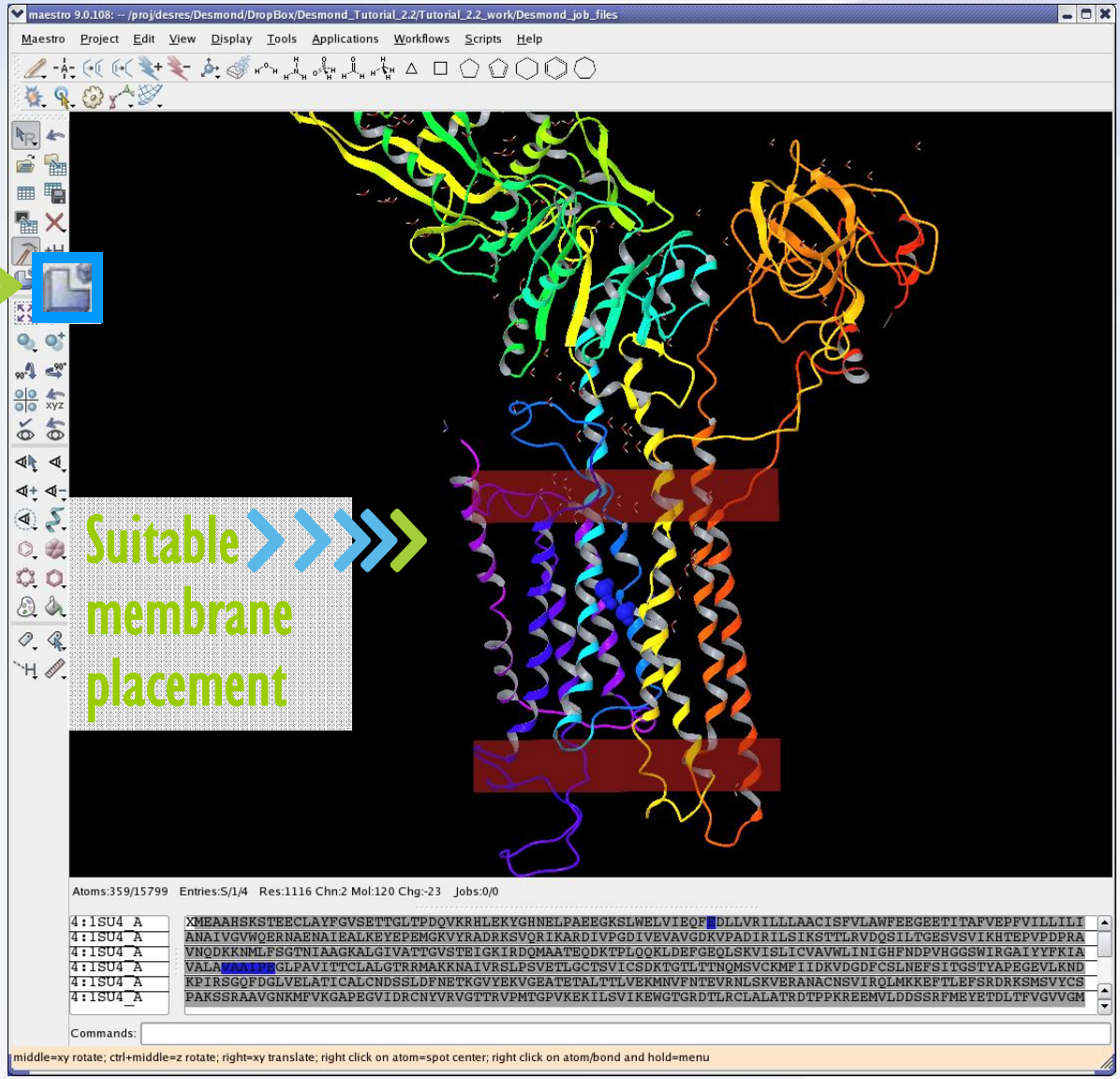
Check the **Adjust membrane position** button in the **Set Up Membrane** window

Translate and rotate the membrane model in the Workspace relative to the protein structure



Setting up membrane systems

Click the **Local transformations** icon to switch between local and global scopes as you adjust membrane position

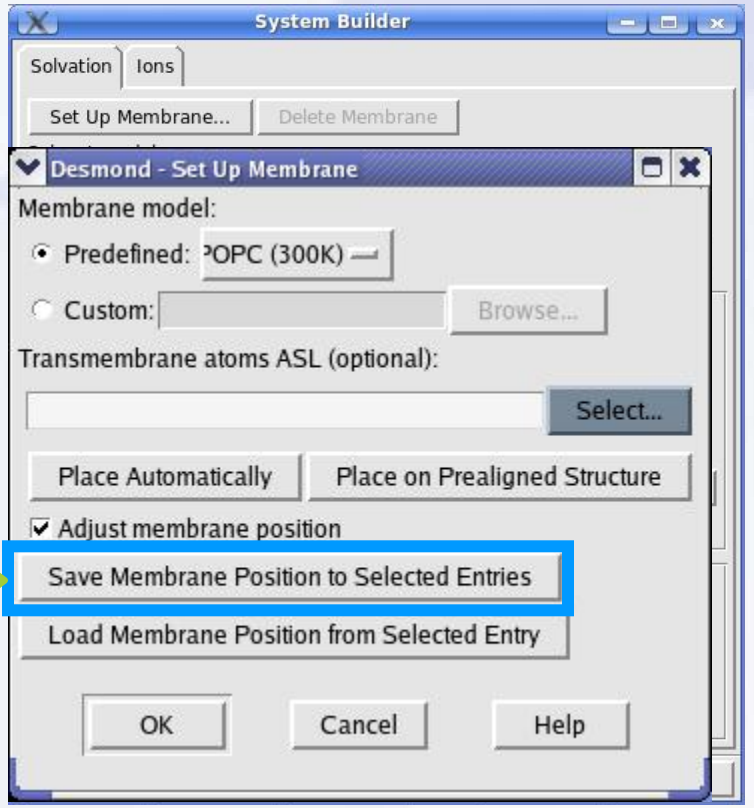

maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Suitable membrane placement
Atoms:359/15799 Entries:S/1/4 Res:1116 Chn:2 Mol:120 Chg:-23 Jobs:0/0
4:1SU4_A
4:1SU4_A
4:1SU4_A
4:1SU4_A
4:1SU4_A
4:1SU4_A
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Setting up membrane systems

Automate membrane placement for positioning two or more membrane proteins exactly the same in the bilayer (e.g. mutagenesis studies)

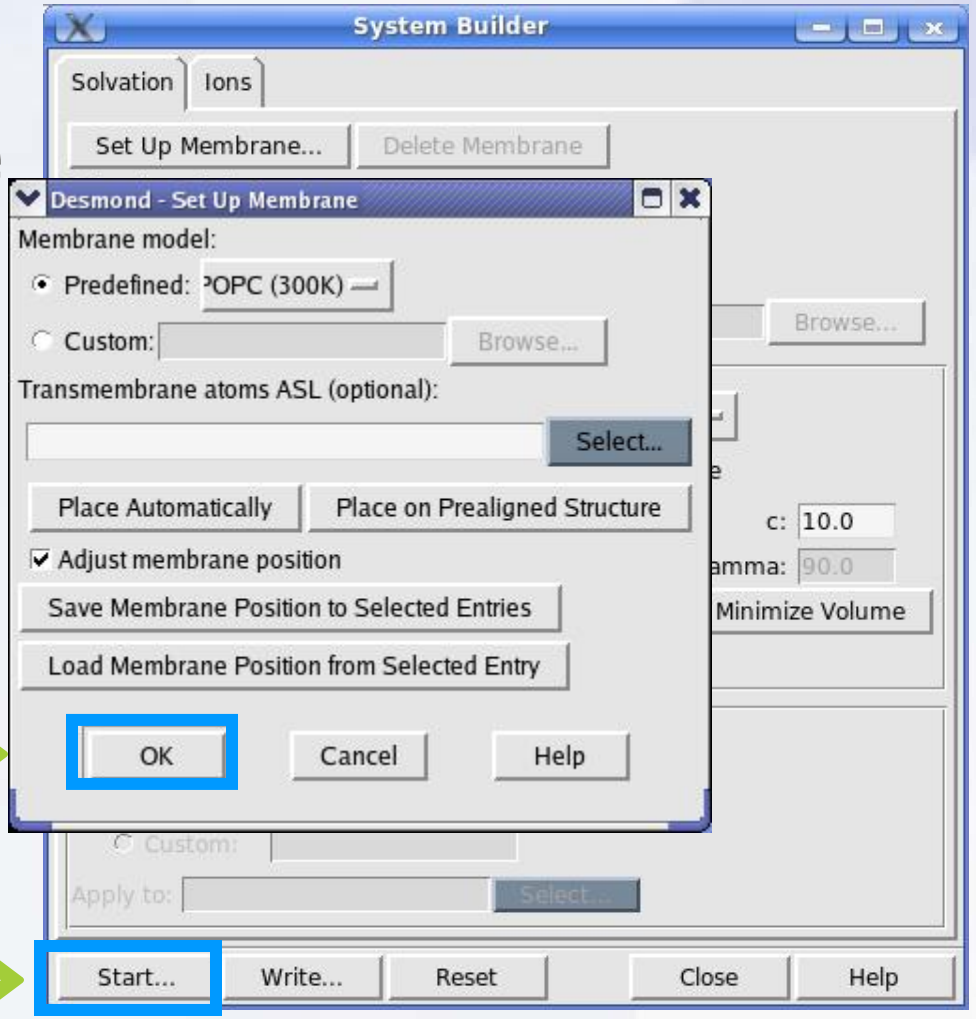
Click the **Save Membrane Position to Selected Entries** button in the **Set Up Membrane** window

For subsequent proteins, click the **Load Membrane Position from Selected Entry** button



Setting up membrane systems

Start the membrane system setup
6-8 minutes to process this example

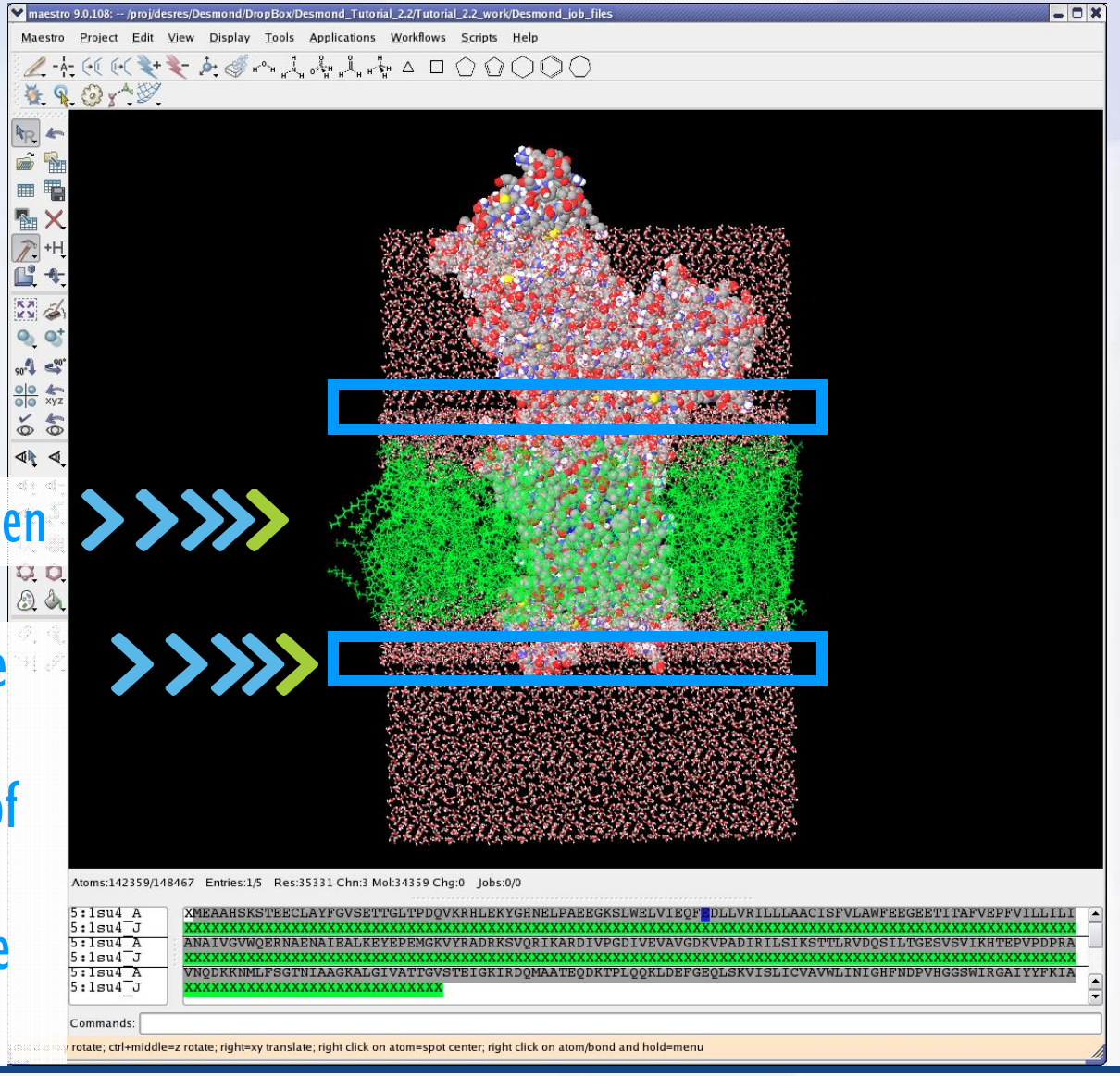


Click **OK** in the **Set Up Membrane** window

Click **Start** in the **System Builder** window

Setting up membrane systems

Resulting simulation system
Protein shown as CPK model


maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:142359/148467 Entries:1/5 Res:35331 Chn:3 Mol:34359 Chg:0 Jobs:0/0
5:1su4_A
5:1su4_J
5:1su4_A
5:1su4_J
5:1su4_A
5:1su4_J
Commands:
rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Lipid bilayer shown in green

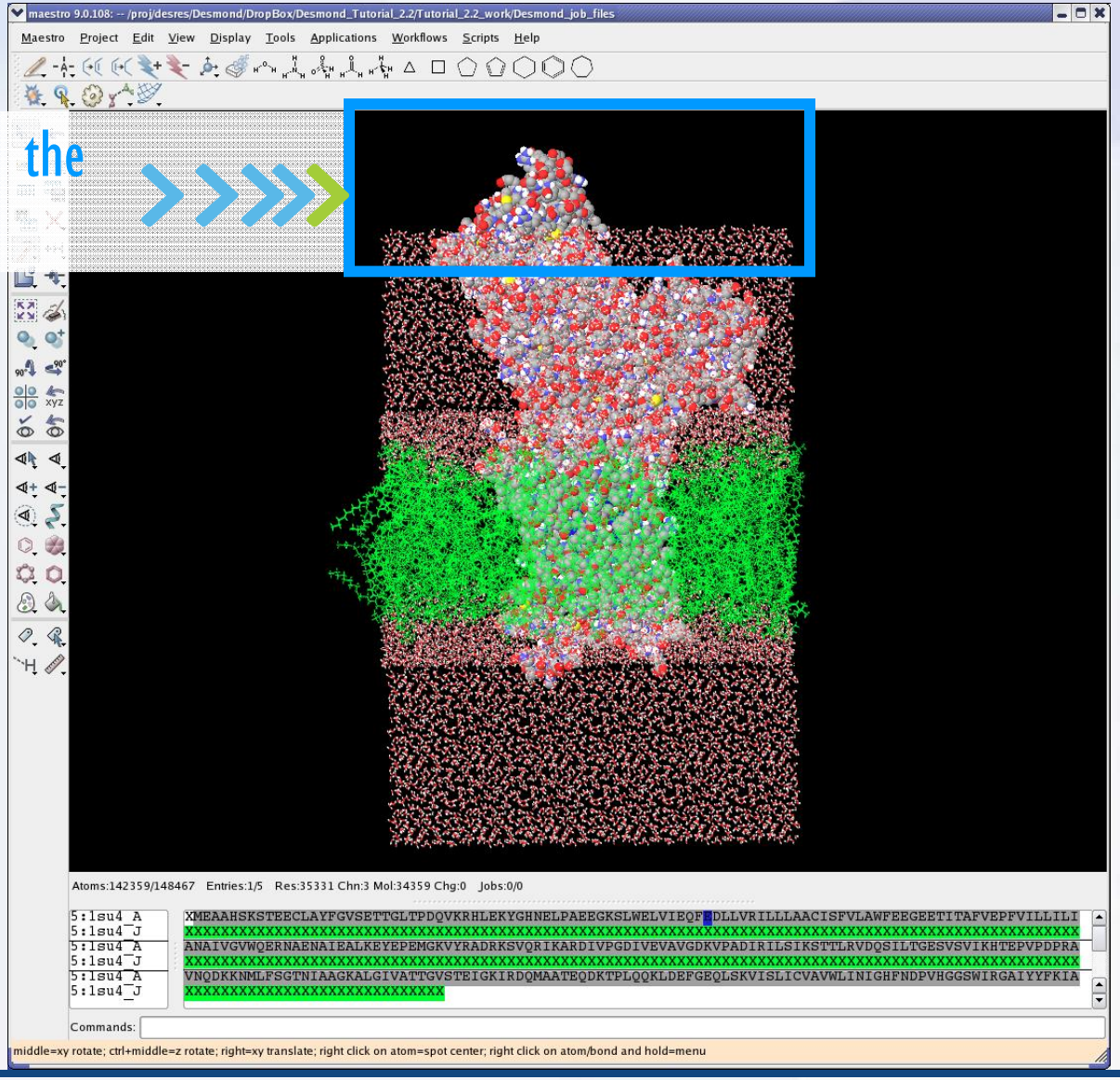
Gaps above and below the lipid bilayer are gaps between the water layer of the POPC model and the added water buffer for the simulation box

Setting up membrane systems

The protein extends above the simulation box

System Builder puts the center of gravity of the solute at the center of the simulation box
Non-spherical systems appear to shift toward one side of the simulation box

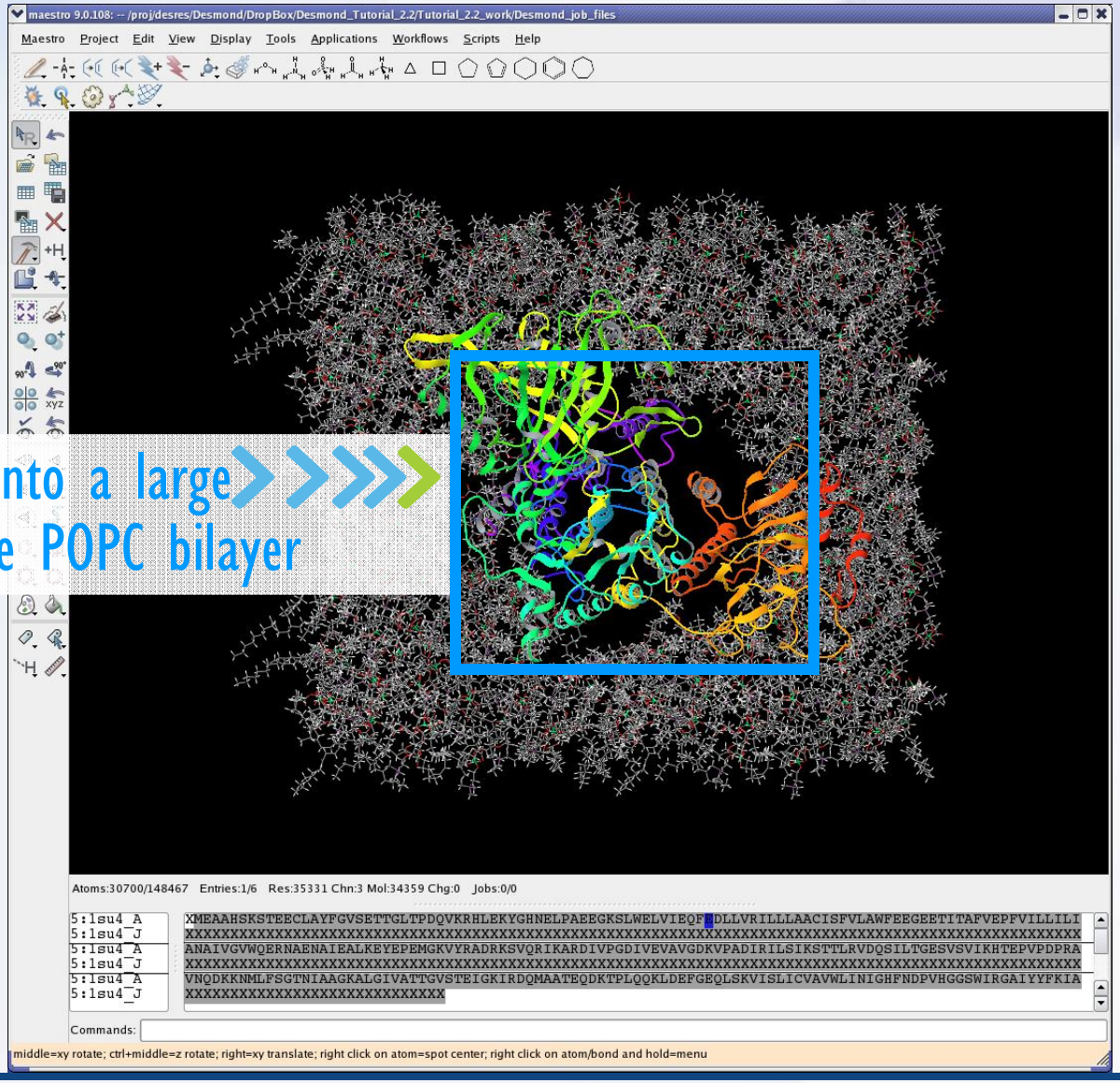
This is a visual artifact, periodic boundary conditions are readily satisfied


maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:142359/148467 Entries:1/5 Res:35331 Chn:3 Mol:34359 Chg:0 Jobs:0/0
5:lsu4_A
5:lsu4_J
5:lsu4_A
5:lsu4_J
5:lsu4_A
5:lsu4_J
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Setting up membrane systems

Membrane systems should be equilibrated for an extended period of time to resolve the transmembrane hole (up to a few hundred ns)

The protein was inserted into a large transmembrane hole in the POPC bilayer

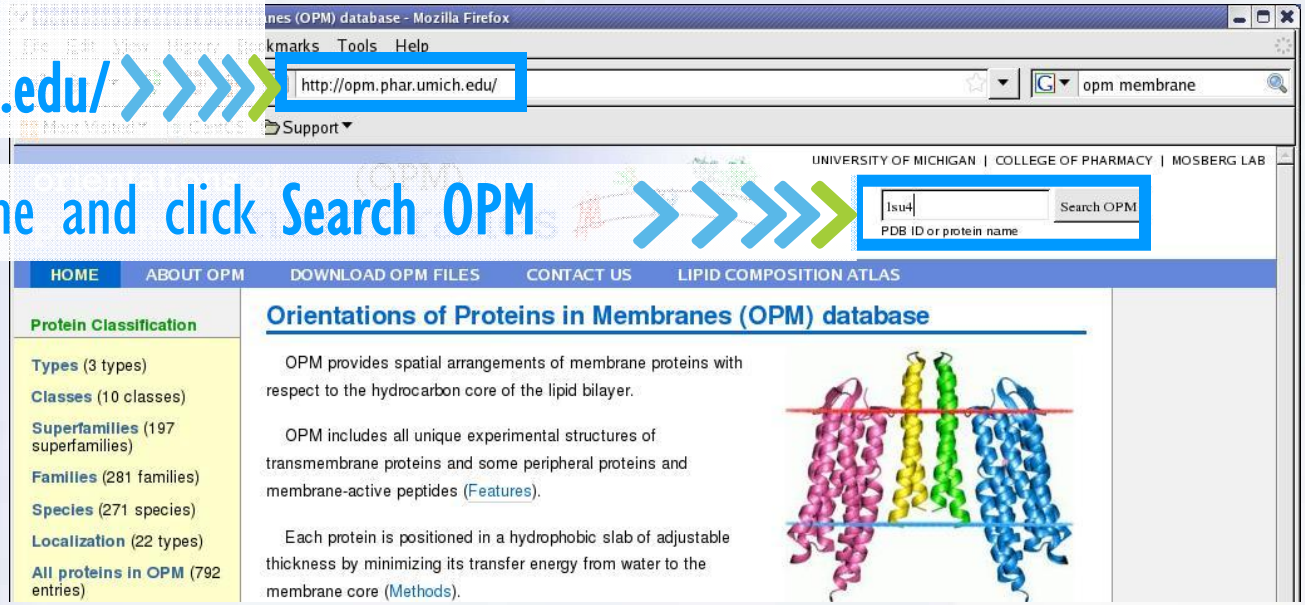

maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:30700/148467 Entries:1/6 Res:35331 Chn:3 Mol:34359 Chg:0 Jobs:0/0
5:1su4_A
5:1su4_J
5:1su4_A
5:1su4_J
5:1su4_A
5:1su4_J
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Importing membrane placement from OPM

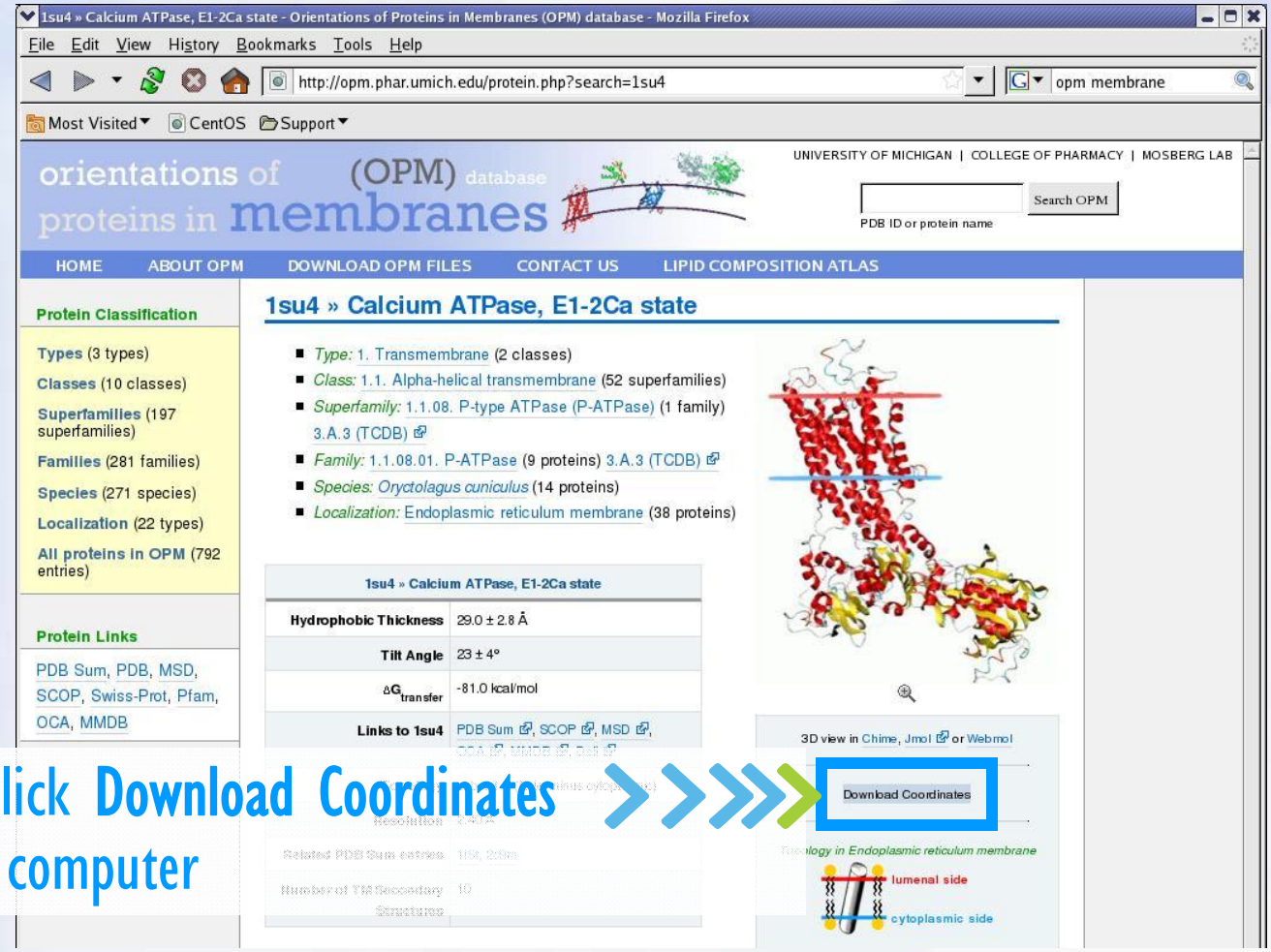
The OPM database at the University of Michigan has high-quality membrane placements

Navigate to http://opm.phar.umich.edu/

Enter the protein name and click Search OPM


nes (OPM) database - Mozilla Firefox
kmarks Tools Help
http://opm.phar.umich.edu/
opm membrane
Support
UNIVERSITY OF MICHIGAN | COLLEGE OF PHARMACY | MOSBERG LAB
1su4
Search OPM
PDB ID or protein name
HOME
ABOUT OPM
DOWNLOAD OPM FILES
CONTACT US
LIPID COMPOSITION ATLAS
Protein Classification
Types (3 types)
Classes (10 classes)
Superfamilies (197 superfamilies)
Families (281 families)
Species (271 species)
Localization (22 types)
All proteins in OPM (792 entries)
Orientations of Proteins in Membranes (OPM) database
OPM provides spatial arrangements of membrane proteins with respect to the hydrocarbon core of the lipid bilayer.
OPM includes all unique experimental structures of transmembrane proteins and some peripheral proteins and membrane-active peptides (Features).
Each protein is positioned in a hydrophobic slab of adjustable thickness by minimizing its transfer energy from water to the membrane core (Methods).

Importing membrane placement from OPM


1su4 » Calcium ATPase, E1-2Ca state - Orientations of Proteins in Membranes (OPM) database - Mozilla Firefox
File Edit View History Bookmarks Tools Help
http://opm.phar.umich.edu/protein.php?search=1su4
opm membrane
Most Visited CentOS Support
orientations of proteins in membranes (OPM) database
UNIVERSITY OF MICHIGAN | COLLEGE OF PHARMACY | MOSBERG LAB
Search OPM
PDB ID or protein name
HOME ABOUT OPM DOWNLOAD OPM FILES CONTACT US LIPID COMPOSITION ATLAS
Protein Classification
Types (3 types)
Classes (10 classes)
Superfamilies (197 superfamilies)
Families (281 families)
Species (271 species)
Localization (22 types)
All proteins in OPM (792 entries)
Protein Links
PDB Sum, PDB, MSD, SCOP, Swiss-Prot, Pfam, OCA, MMDB
1su4 » Calcium ATPase, E1-2Ca state
Type: 1. Transmembrane (2 classes)
Class: 1.1. Alpha-helical transmembrane (52 superfamilies)
Superfamily: 1.1.08. P-type ATPase (P-ATPase) (1 family)
3.A.3 (TCDB)
Family: 1.1.08.01. P-ATPase (9 proteins) 3.A.3 (TCDB)
Species: Oryctolagus cuniculus (14 proteins)
Localization: Endoplasmic reticulum membrane (38 proteins)
1su4 » Calcium ATPase, E1-2Ca state
Hydrophobic Thickness 29.0 ± 2.8 Å
Tilt Angle 23 ± 4°
ΔG transfer -81.0 kcal/mol
Links to 1su4 PDB Sum, SCOP, MSD,
3D view in Chime, Jmol or Webmol
Download Coordinates
lumenal side
cytoplasmic side

In the results page, click **Download Coordinates**

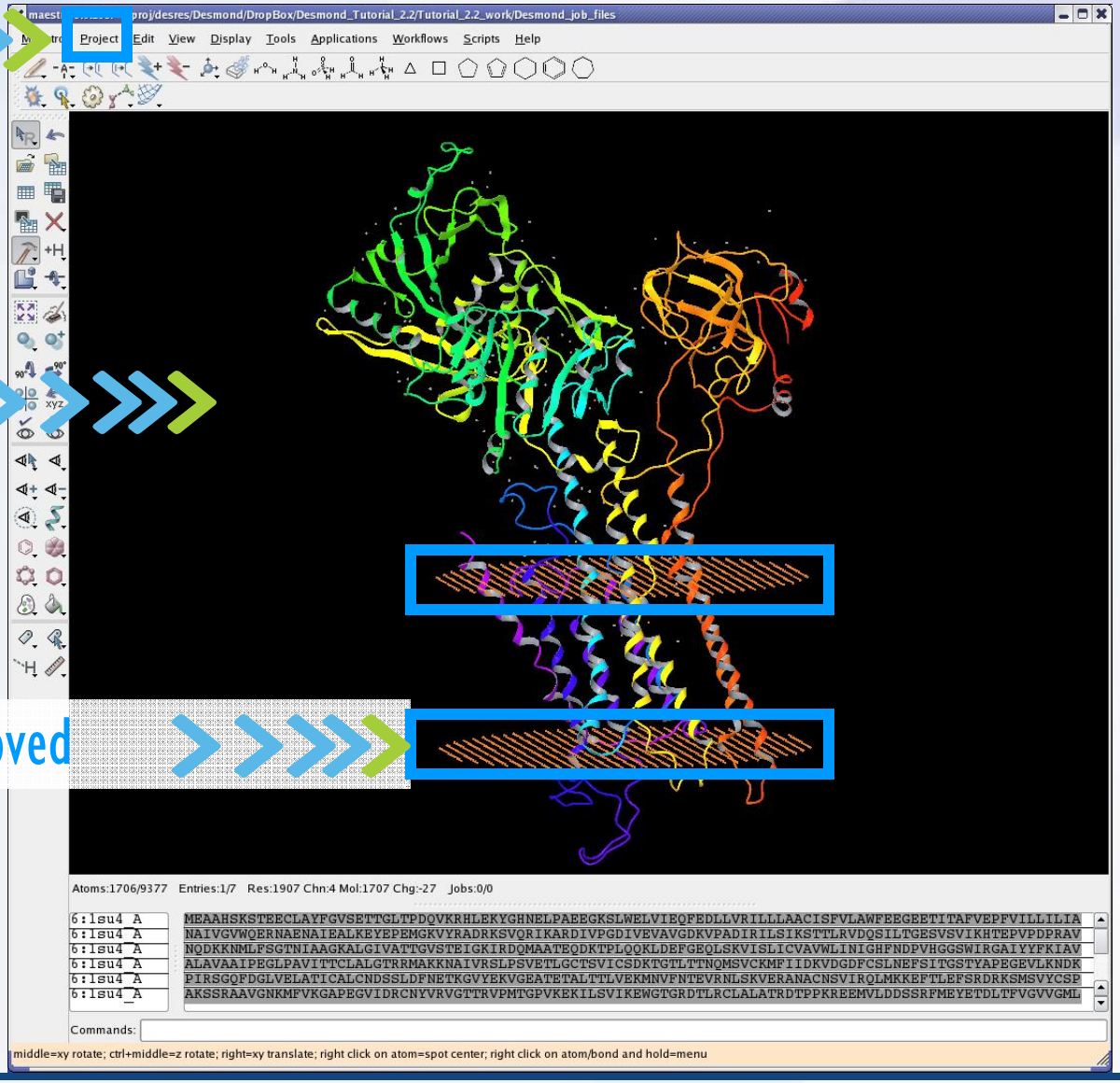
Save the file to your computer

Importing membrane placement from OPM

Select Project >Import Structures

The system is shown in a similar orientation to the one we used before

Dummy atoms to be removed



Importing membrane placement from OPM

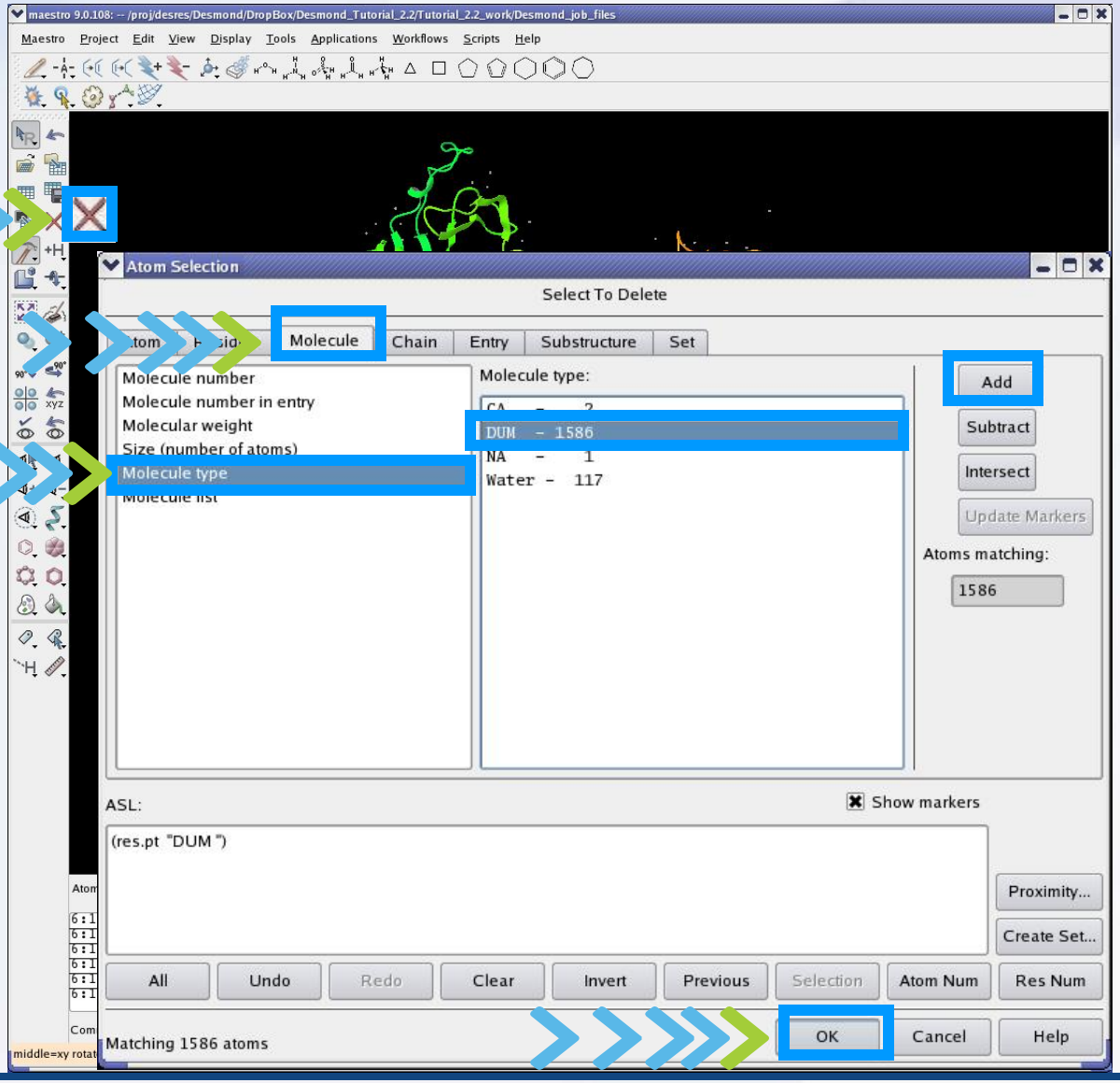
Remove dummy atoms

Click the **X** icon

Click the **Molecule** tab

Select **Molecule type**
and select **DUM**
and click the **Add** button

Click **OK**



Importing membrane placement from OPM

Preprocess with the Protein Preparation Wizard

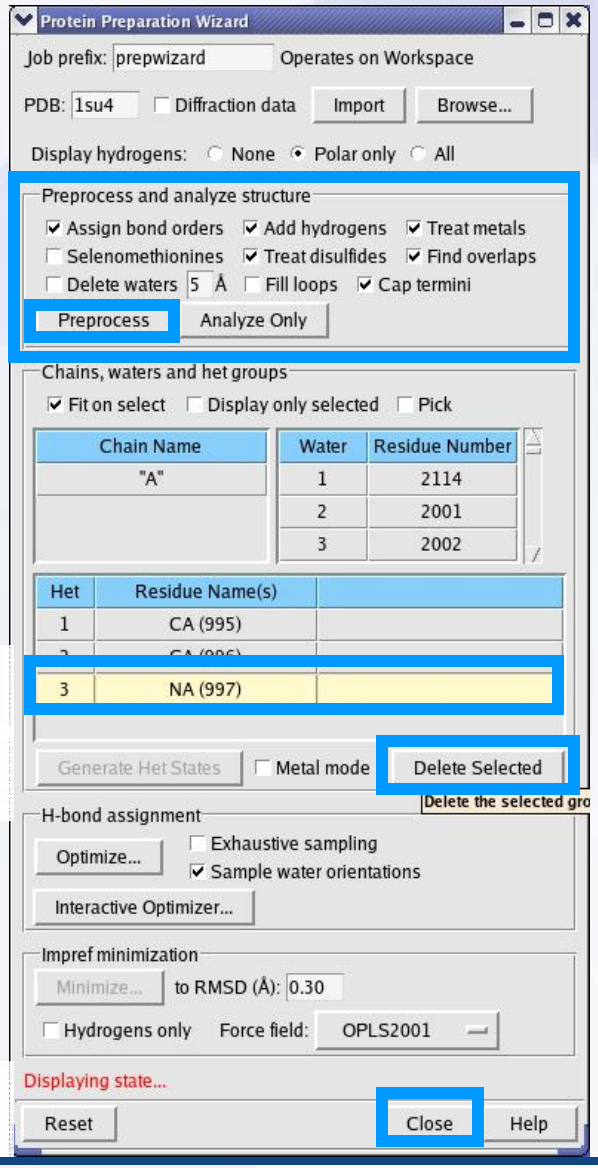
Use the structure in the Workspace

Check options for preprocessing as shown
(do not delete crystal waters)

Click Preprocess

Select the sodium ion in the Het table
Click the Delete Selected button

Click Close



Importing membrane placement from OPM

Select **Applications>Desmond>System Builder**

Click the **Ions** tab

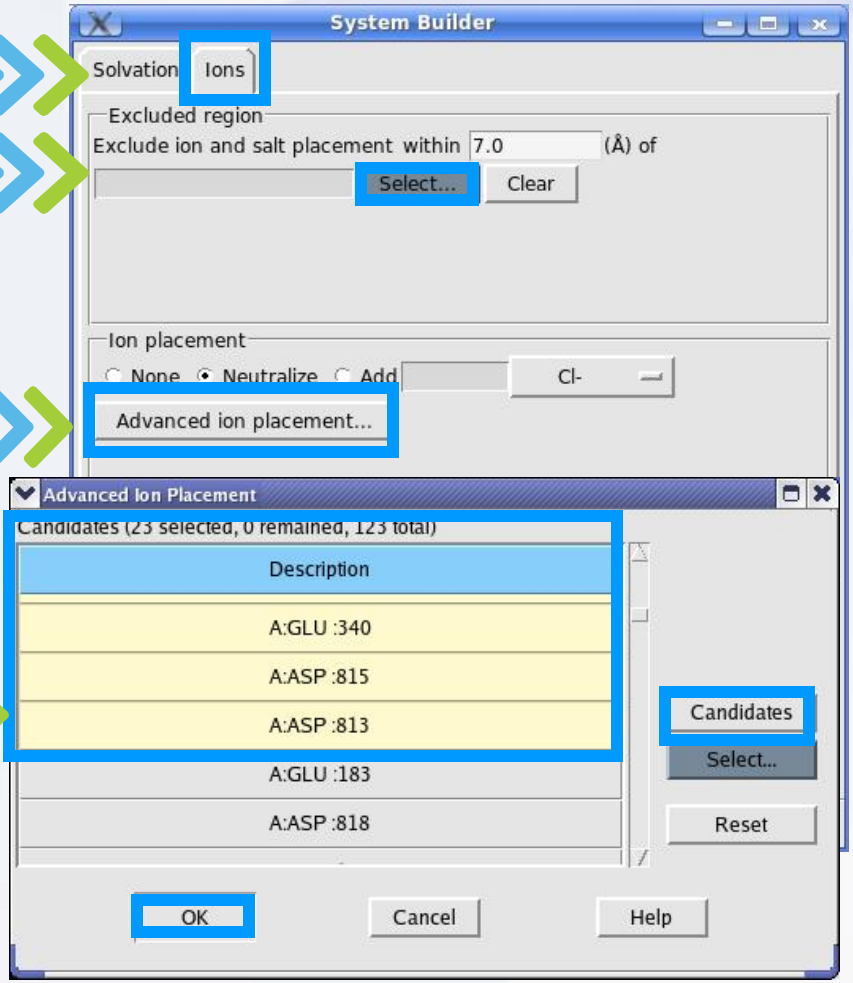
Click the **Select** button

Click the **Advanced ion placement** button

Click the **Candidates** button

Shift-click to select the first 23 residues under **Candidates**

Click **OK**



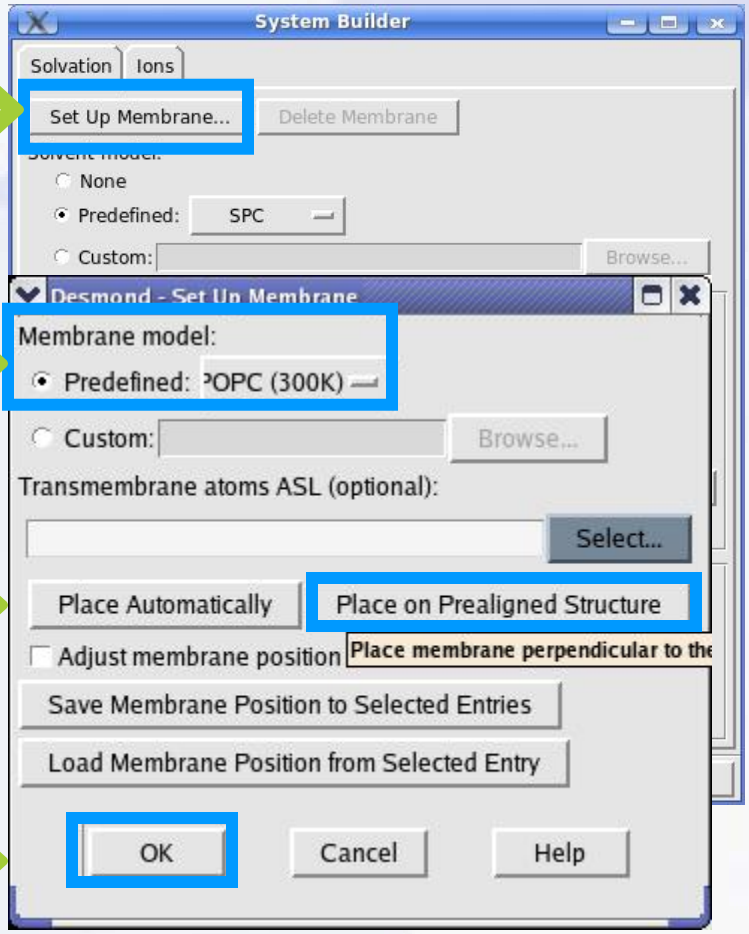
Importing membrane placement from OPM

Click the **Set Up Membrane** button

Select **POPC** as the membrane model

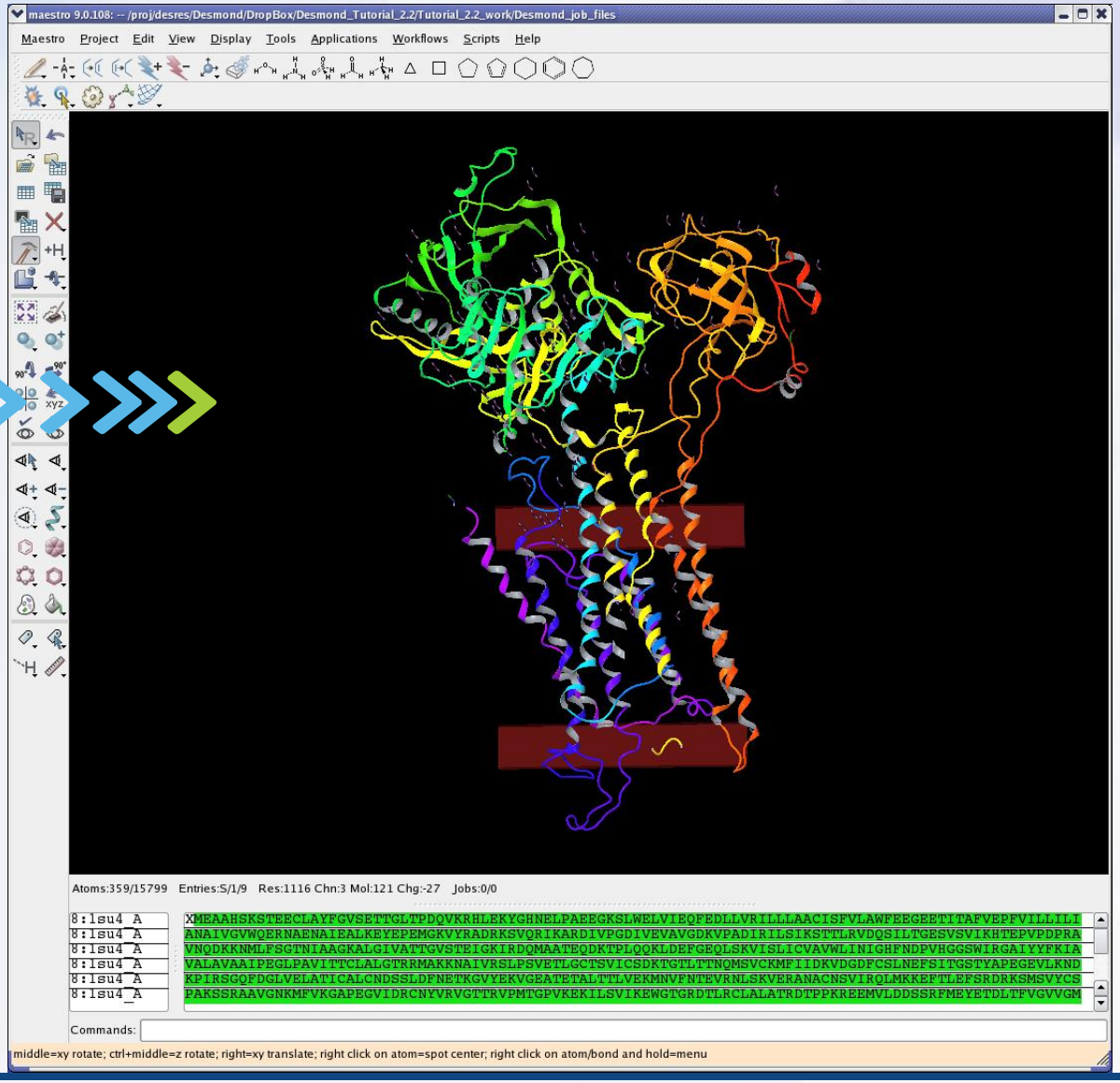
Click the **Place on Prealigned Structure** button

Click **OK**



Importing membrane placement from OPM

The OPM pre-aligned membrane is shown in the Workspace


maestro 9.0.108: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:359/15799 Entries:S/1/9 Res:1116 Chn:3 Mol:121 Chg:-27 Jobs:0/0
8:lsu4_A
8:lsu4_A
8:lsu4_A
8:lsu4_A
8:lsu4_A
8:lsu4_A
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

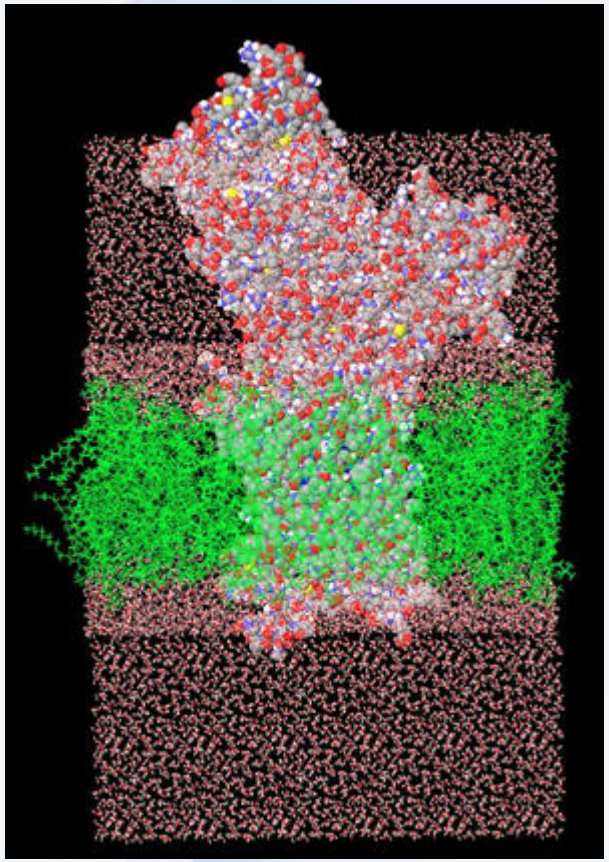
Importing membrane placement from OPM

Our manual membrane placement

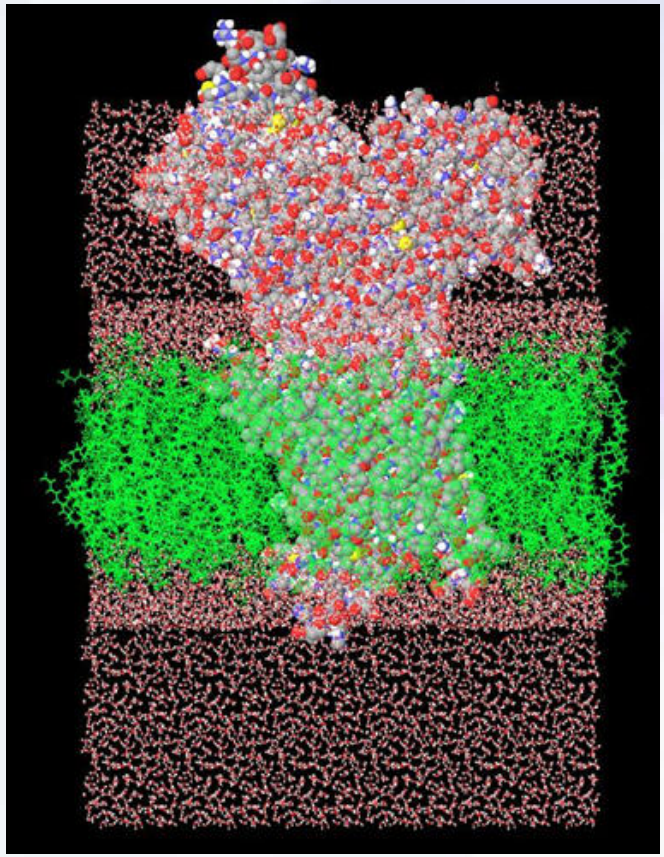
OPM membrane placement

Importing membrane placement from OPM

Click **Start** in the **System Builder** to generate the membrane

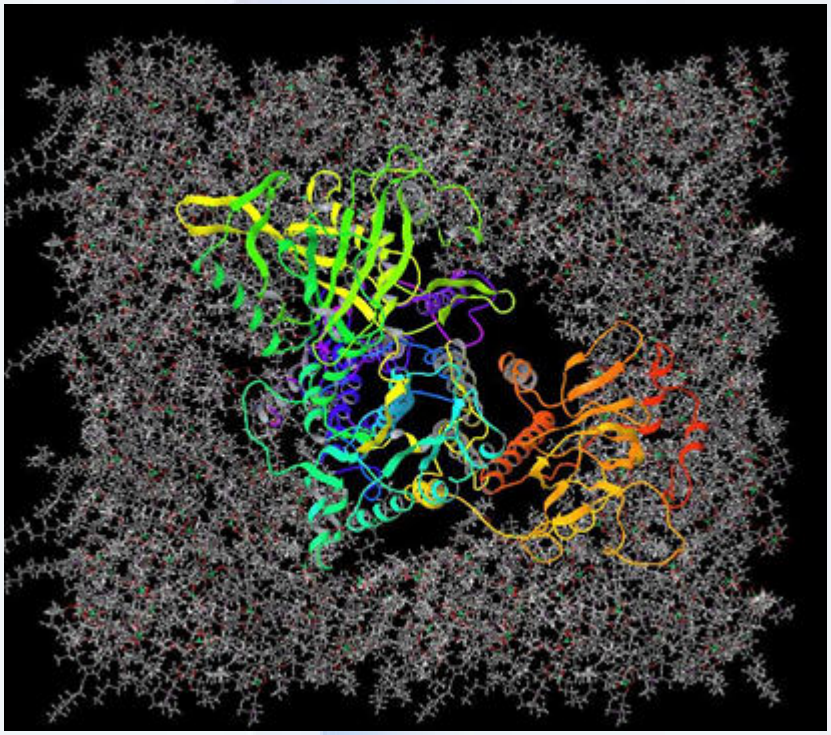


Our full membrane simulation system

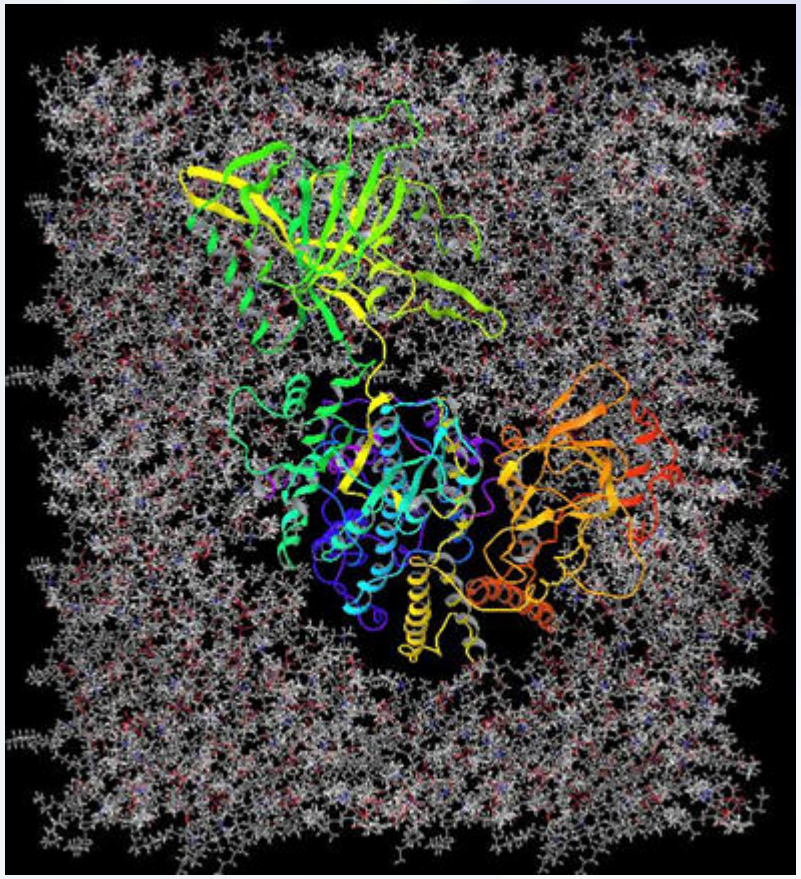


OPM full membrane simulation system

Importing membrane placement from OPM



Our transmembrane hole



OPM transmembrane hole

Generating force field parameters with Viparr

- Assign alternative force field parameters with ***Viparr***
- Viparr is a Python script that reads .cms (or .mae) files
- Refers to a template database to assign force field parameters
- Matches residues to templates using atomic numbers & bond structure
- Built-in force fields include latest versions of Amber, CHARMM, and OPLS-AA for fixed charge model and *pff* polarizable force field
- Wipes out existing `ffio_ff{ }` structure in the .cms input file
- Does not alter atom or residue ordering when creating the output file
- **Recommended:** perform post-processing on .cms with Viparr
- Invoke from command line:

```
$SCHRODINGER/run –FROM desmond viparr.py --help
```

Generating force field parameters with Viparr

- Can specify multiple force fields
- Example:

```
$ viparr.py –f charmm27 –f tip4p 1su4_desmond.cms
1su4_desmond_out.cms
```

- Combine components of two or more force fields
- All matching force fields are applied
- Conflicting parameters: first force field in command line invocation takes precedence
- Viparr aborts if a bond exists between two residues that are not matched by the same force field, or if force fields have inconsistent van der Waals combining rules

Generating force field parameters with Viparr

- Viparr generates error or warning messages when:
  - A residue matches more than one template in a force field
  - A residue name is matched to a force field template with a different name
  - There are unmatched residues
  - A residue is matched by more than one of the selected force fields

Generating force field parameters with Viparr

- **-c** option allows you to assign parameters for a particular structure (`f_m_ct{ }` block) in the input **.cms** file
- **-n** option allows you to include comments in the output **.cms** file for annotation purposes
- You must add constraints in a separate step with Viparr:

```
$ $SCHRODINGER/run – FROM desmond
build_constraints.py input.cms output.cms
```

where **input.cms** is the Maestro file with Viparr parameters and **output.cms** includes the constraints

Importing AMBER simulation systems

- Simulation systems from the Amber Molecular Dynamics package can be imported to Desmond
- Desmond can convert **prmtop** and **crd** files into **cms** file
- Script is called **amber_prm2cms**

```
$SCHRODINGER/run –FROM mmshare amber_prm2cms.py
–p my_amber_system.prm –c my_amber_system.crd
-o output.cms
```

- Desmond applies force field parameters in one-to-one agreement with the Amber **prmtop** file
- Before simulation add constraints with **build_constraints** script

Desmond simulation parameters

- Set simulation parameters by:
  - Using Desmond applications in Maestro (**Applications>Desmond**)
  - Editing the Desmond configuration file
- Applications include:
  - Minimization
  - Molecular dynamics
  - Simulated annealing
  - Free energy perturbation (FEP)
  - Replica exchange

Desmond simulation parameters

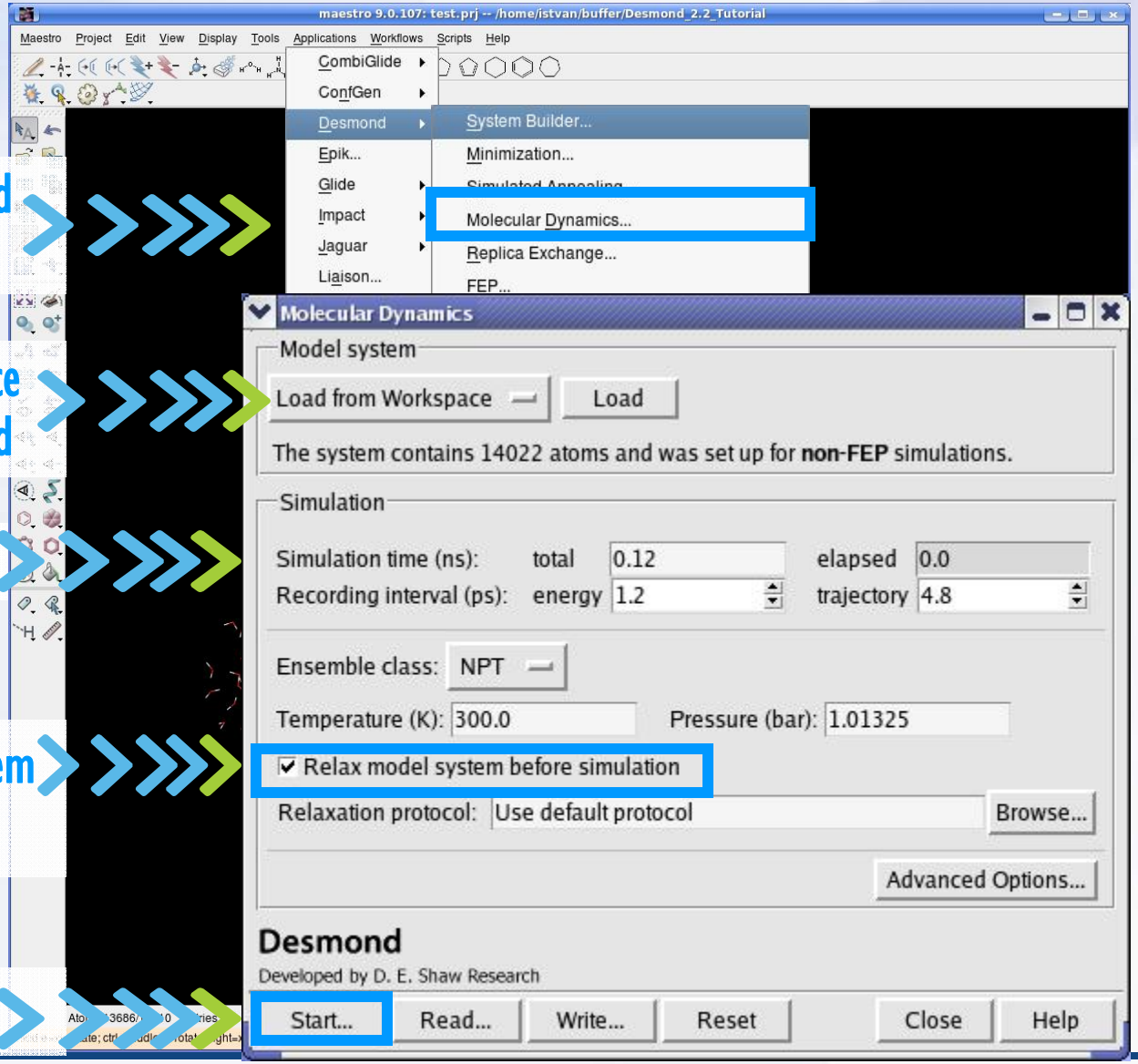
Select Applications>Desmond >Molecular Dynamics

Choose Load from Workspace or Load from file, click Load

Set simulation options

Select the Relax model system before simulation option

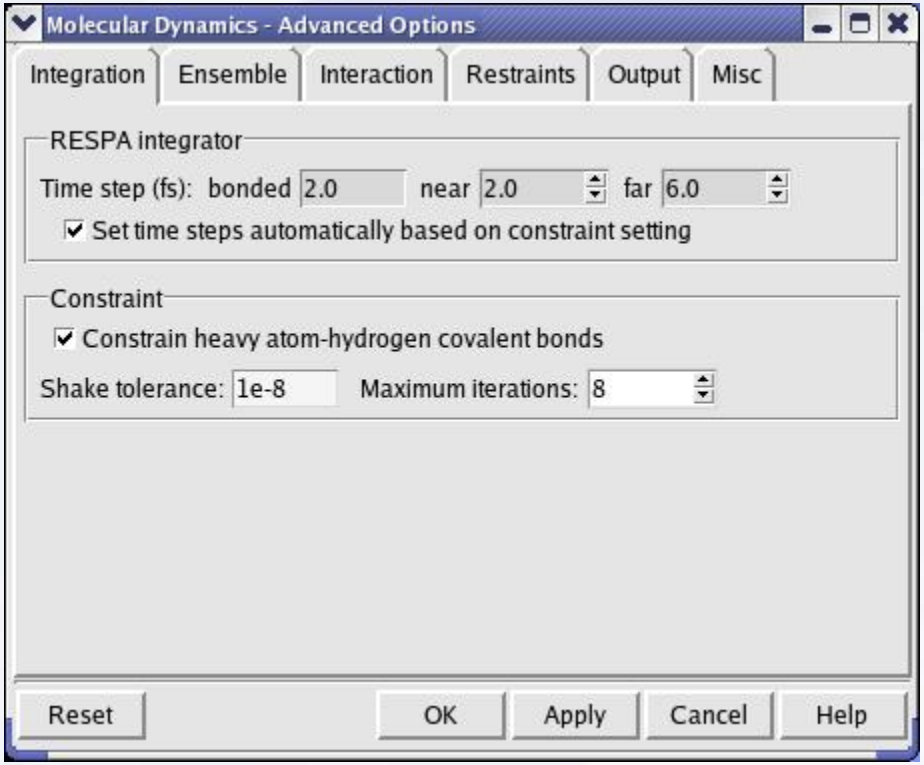
Click Start



Desmond simulation parameters

Click **Advanced Options**

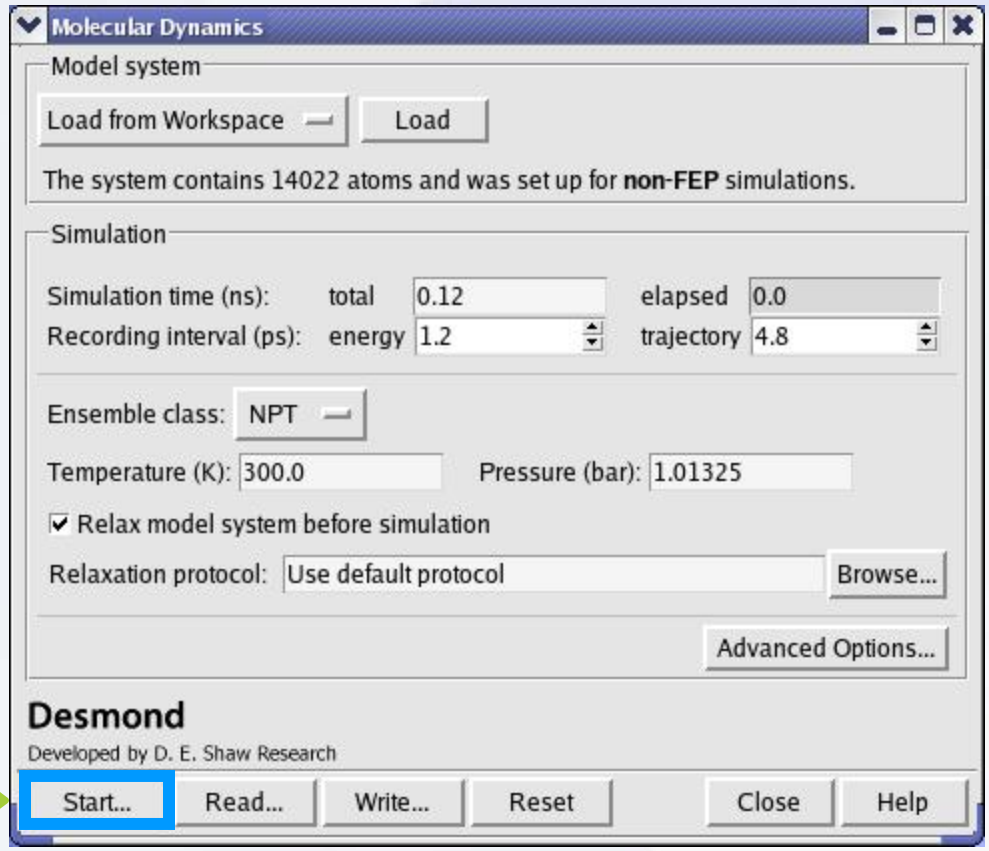
- **Integration**—Set multiple time step parameters and constraints
- **Ensemble**—Set thermostat and barostat parameters
- **Interaction**—Set parameters for computing non-bonded interactions
- **Restraint**—Subject multiple sets of atoms to harmonic restraint with a tunable force constant
- **Output**—Control output file names and specify how often files are updated during simulation
- **Misc**—Set miscellaneous parameters including the definition of atom groups or the construction of trajectory files



Running Desmond simulations

- Run Desmond simulations by:
  - Clicking **Start** in the **Molecular Dynamics** window
  - Entering commands at the command line
- Structure relaxation prepares the system for production-quality MD simulation
- Maestro's relaxation protocol includes:
  - 2 stages of minimization (restrained and unrestrained)
  - 4 stages of short MD runs with gradually diminishing restraints and increasing temperature

Running Desmond simulations



Click **Start**

Running Desmond simulations

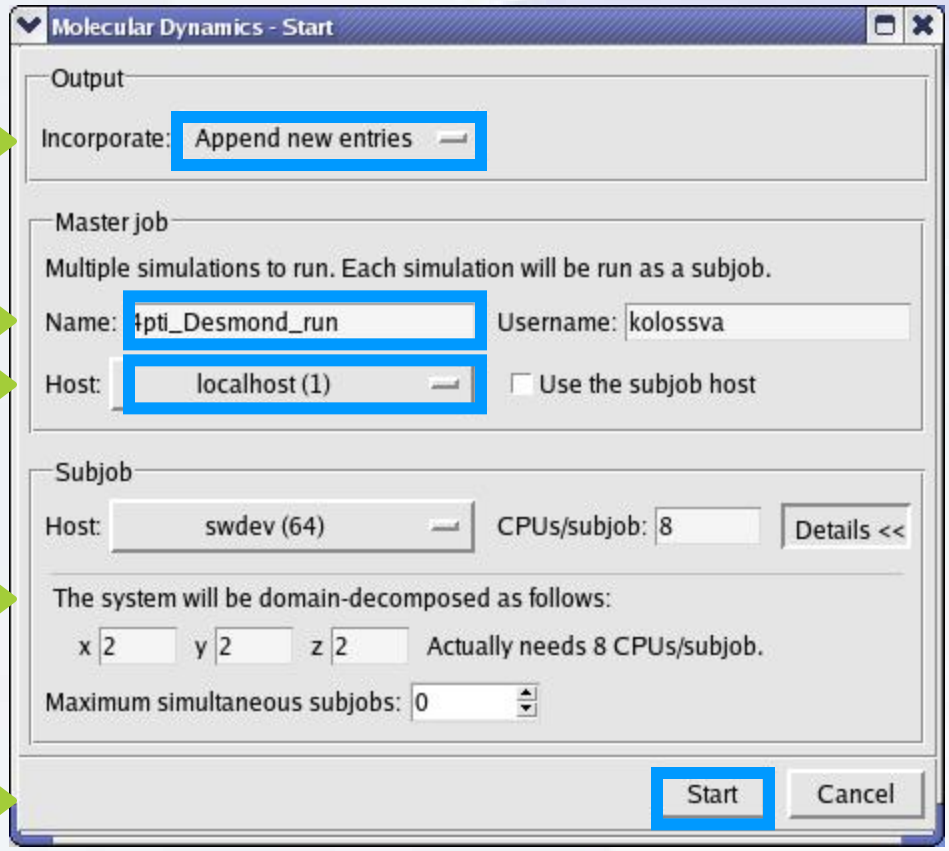
Select **Append new entries** to add results to the current project

Enter a name for the job

Select the host where the master job should run; usually **localhost**

Set job submission parameters

Click **Start**



Running Desmond simulations

- Run simulations from the command line:

```
$SCHRODINGER/desmond -HOST <hostname>
-exec <desmond_task> -P <cpu> -c <jobname>.cfg
–in <jobname>.cms
```

- where:
  - `desmond_task` can be `minimize` (simple minimization), `mdsim` (MD simulation), `vrun` (trajectory analysis)
  - `jobname` is the name of the job
  - `hostname` is the name of the compute host or queue on a cluster
  - `cpu` specifies the number of CPUs you want each Desmond simulation subjob to use (either 1 for quick tests or a power of 2)

Running Desmond simulations

- Advanced issues to consider:
  - The Desmond driver tries automatically to optimize configuration parameters; turn this off using the `-noopt` argument
  - Automatically generate Desmond configuration settings for efficient force computations with the **forceconfig** script:
    `$SCHRODINGER/run -FROM desmond forceconfig.py <input.cms>`
  - While a Desmond job is running, all files for the job are continuously updated in a temporary directory:
    tmpdir/username/jobname
    where **tmpdir** is specified in **$SCHRODINGER/schrodinger.hosts**
    Use `-LOCAL` option to keep job files in the working directory

Running Desmond simulations

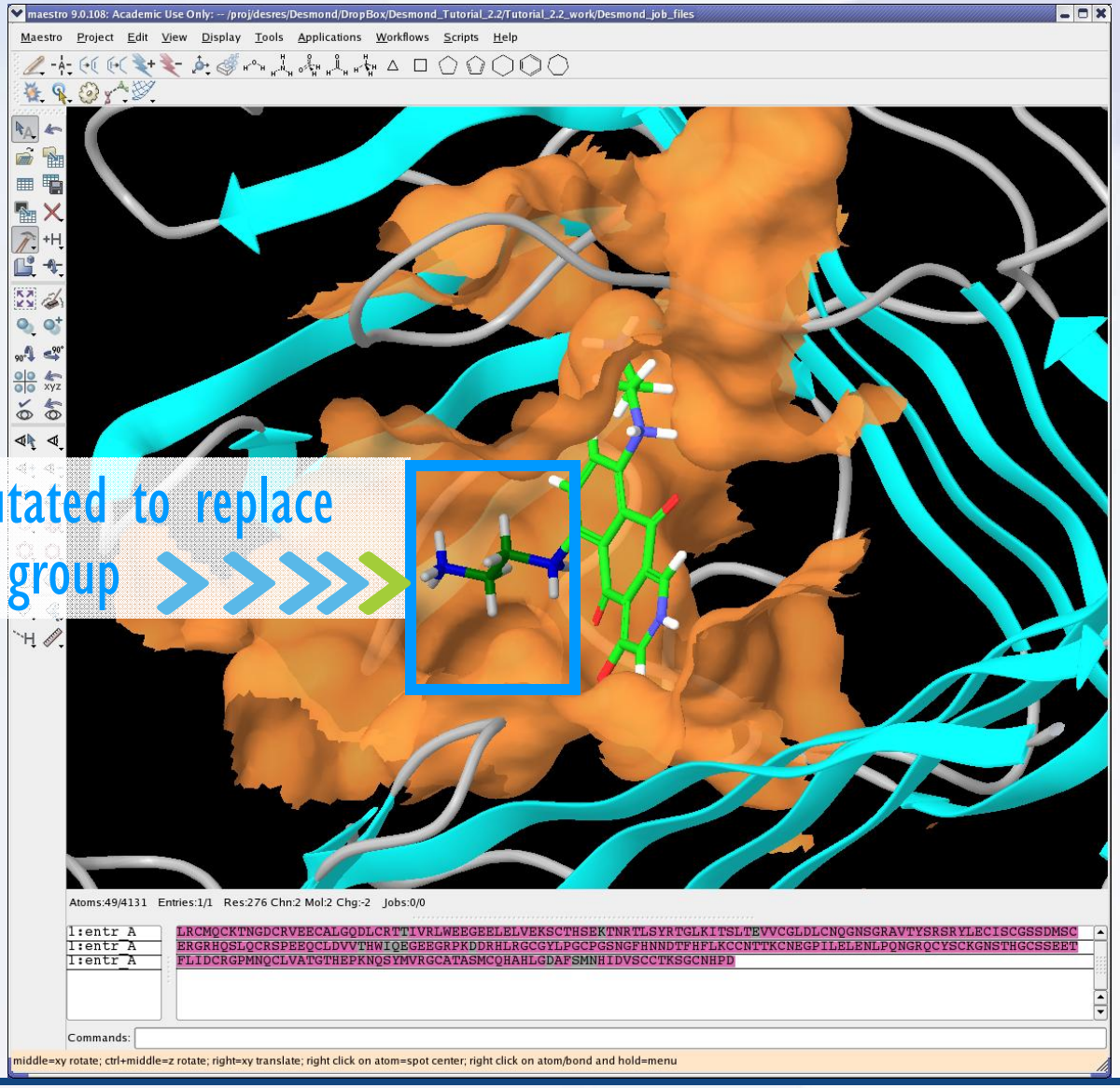
- Run multiple jobs from the command line
  - May be necessary for system relaxation and FEP simulations
  - Multiple jobs are handled by the **MultiSim** facility
  - Reads a **.msj** command script file and runs simulations defined within it
- `$SCHRODINGER/utilities/multisim -JOBNAME <jobname> -maxjob <maxjob> -cpu <cpu> -HOST localhost -host <hostname> -i <jobname>.cms -m <jobname>.msj -o <jobname>-out.cms`
  - `maxjob` is the maximum # of simultaneous subjobs (0=no limit)
  - `cpu` is the # of CPUs per subjob (8 CPUs can be "2 2 2")
  - `hostname` is the host or queue where simulation jobs will run

Preparing free energy perturbation

- Desmond can run free energy perturbation (FEP) calculations:
  - Ligand and amino acid residue mutation
  - Annihilation (absolute free energy calculation)
  - Single-atom mutation in a ring structure
- Visually specify fixed and variable parts of a molecule for defining the mutation
- Store the atom correspondence map in the **.mae** file (dual topology)

Preparing free energy perturbation

Example ligand mutation

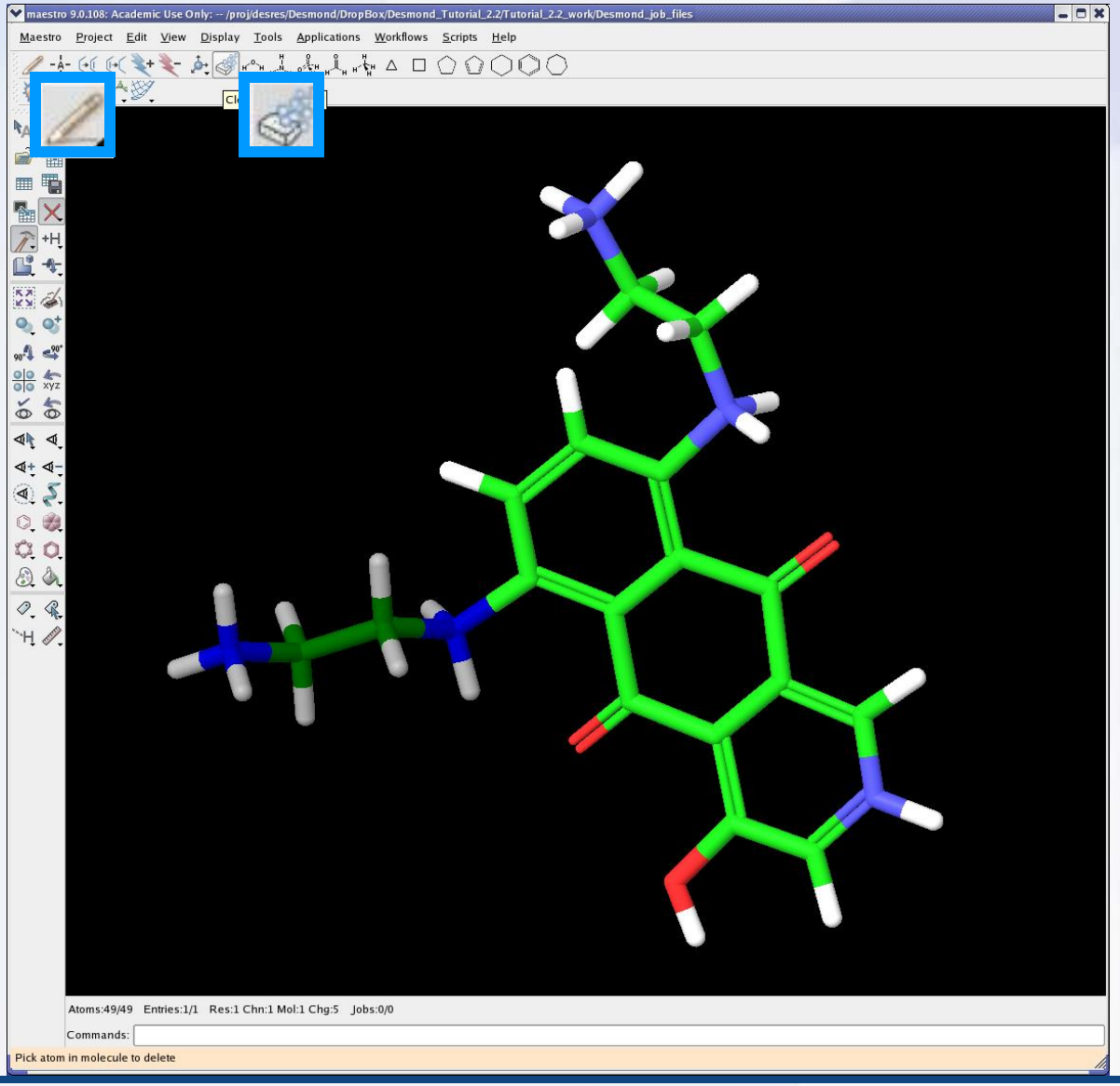

maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
This side chain will be mutated to replace the NH3+ with a methyl group
Atoms:49/4131 Entries:1/1 Res:276 Chn:2 Mol:2 Chg:-2 Jobs:0/0
1:entr_A LRCMQCKTNGDCRVEECALGQDLCRTTIVRLWEEGEELELVEKSCTHSEKTNRTLSYRTGLKITSLTEVVCGLDLCNQGNSGRAVTYSRSRYLECISCGSSDMSC
1:entr_A ERGRHQSLQCRSPEEQCLDVVTHWIQEGEEGRPKDDRHLRGCGYLPGCPGSNGFHNNDTFHFLKCCNTTKCNEGPILELENLPQNGRQCYSCKGNSTHGCSSEET
1:entr_A FLIDCRGPMNQCLVATGTHEPKNQSYMVRGCATASMCQHAHLGDAFSMNHIDVSCCTKSGCNHPD
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Preparing free energy perturbation

Build the ligand structure using hand-drawing and geometry-cleanup options

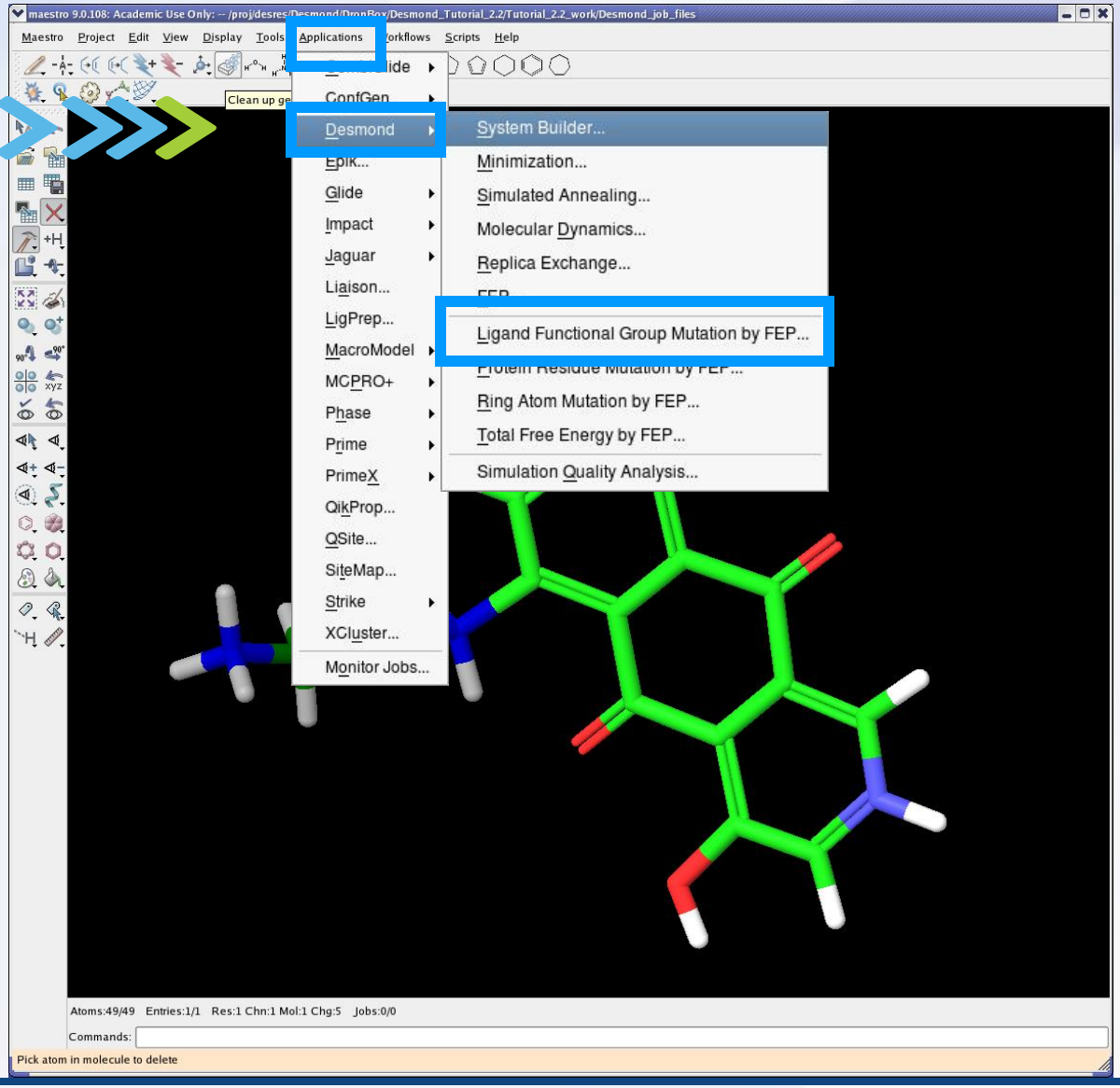
Click the **Pencil** icon to draw a ligand structure

Click the **Clean Up** icon to automatically correct the structure to give reasonable 3-D geometry


maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:49/49 Entries:1/1 Res:1 Chn:1 Mol:1 Chg:5 Jobs:0/0
Commands:
Pick atom in molecule to delete

Preparing free energy perturbation

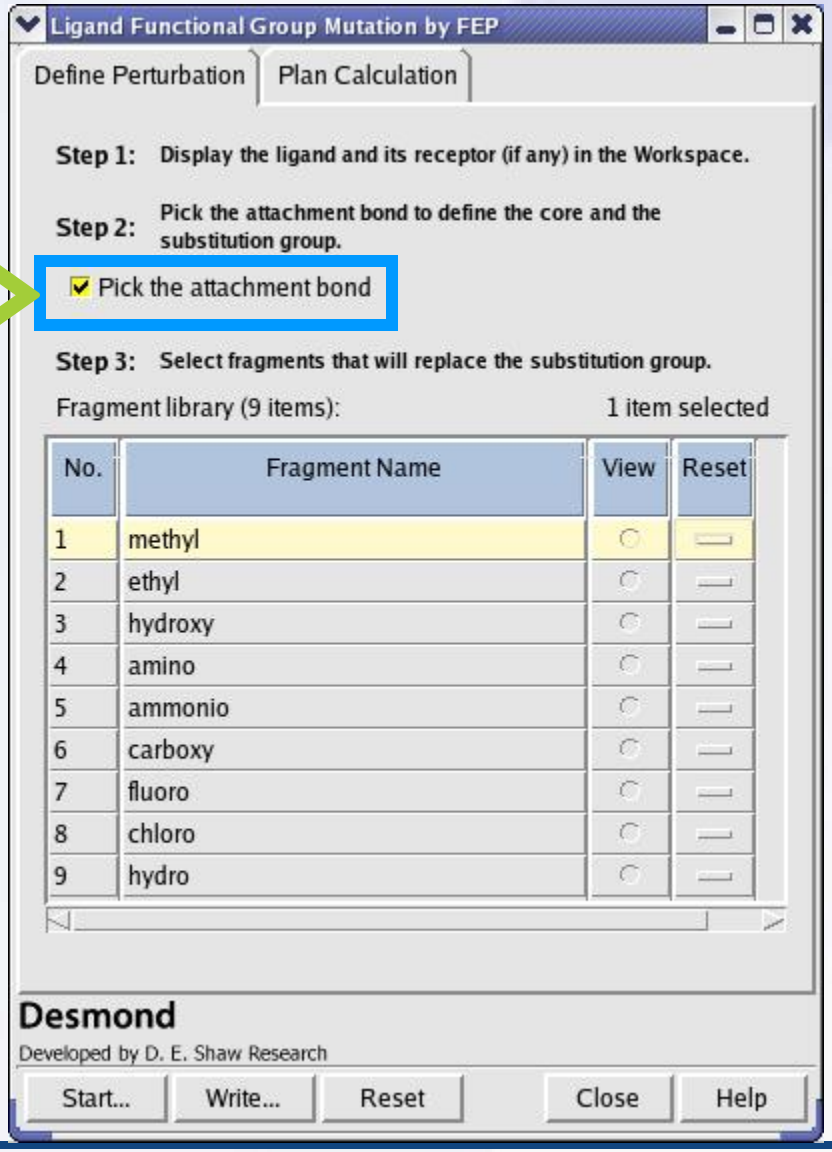
Select Applications> Desmond>Ligand Functional Group Mutation by FEP…



Preparing free energy perturbation

Select the **Pick the attachment bond** option

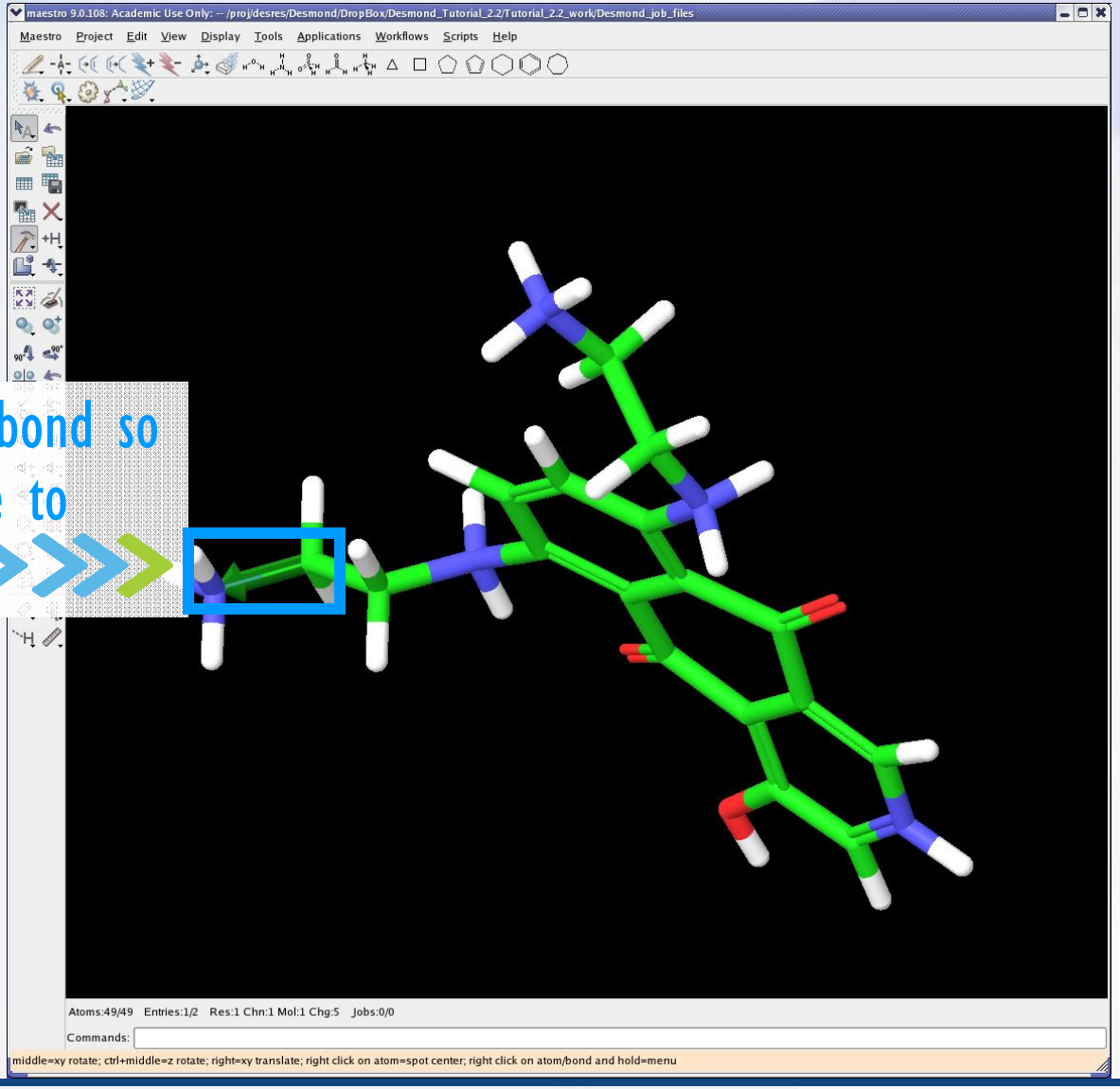
The core and substitution groups are connected by a single bond called the *attachment bond* (green arrow)



Preparing free energy perturbation

Hand-pick the attachment bond so the arrow points from core to substitution group >>>>>

You determine the direction of the green arrow based on which half of the attachment bond you select

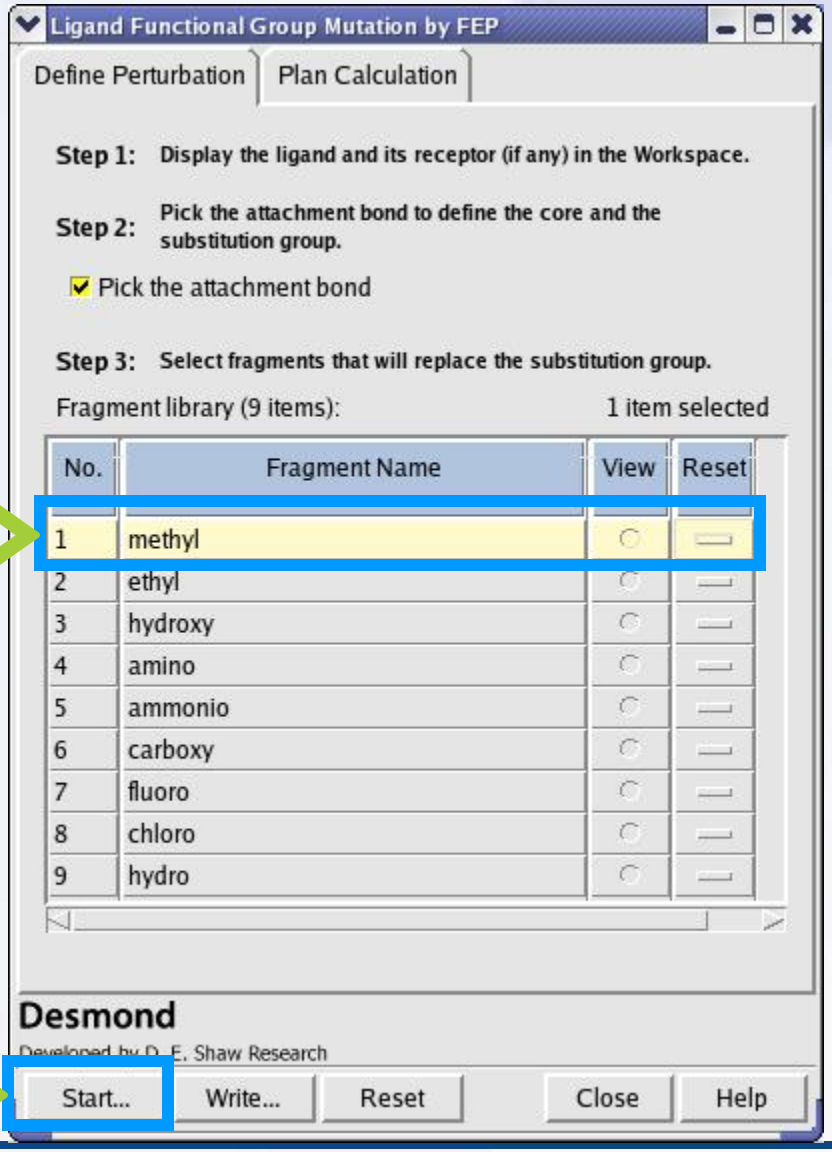

maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:49/49 Entries:1/2 Res:1 Chn:1 Mol:1 Chg:5 Jobs:0/0
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Preparing free energy perturbation

Select the fragment that will replace the substitution group

For multiple fragments, FEP creates different systems for each mutant and computes binding free energy difference between reference ligand and each selected mutant, with respect to the receptor

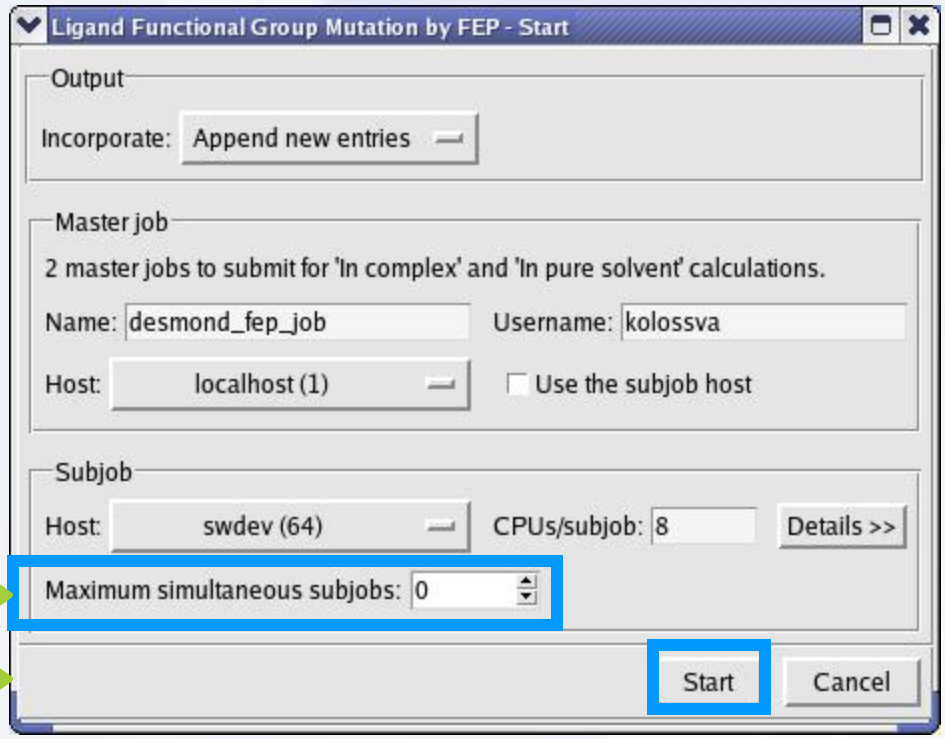
Click **Start**



Preparing free energy perturbation

Desmond uses Bennett's acceptance ratio method for FEP calculations

Numerous independent "Lambda window" simulations will be run for a single FEP calculation



Control how many simulations can run simultaneously
0 = no limit

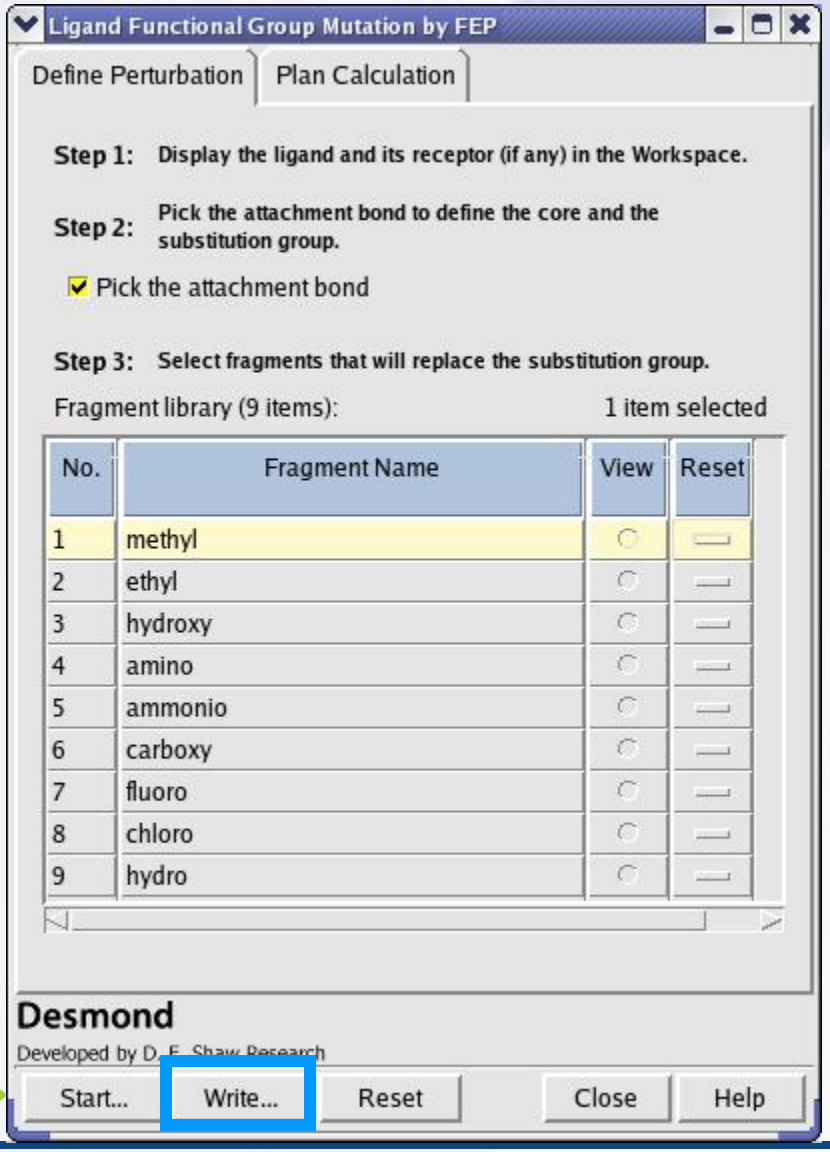
Click **Start**

Preparing free energy perturbation

If you click **Write**, these files are written:

- **my-fep-job_lig.mae** contains the original ligand and one or multiple mutants
- **my-fep-job_cmp.mae** contains the receptor and ligand structures
- **my-fep-job_solvent.msj** defines a series of simulations involving the ligands in the solvent
- **my-fep-job_complex.msj** defines corresponding simulations involving the ligand-receptor complexes

Click **Write**



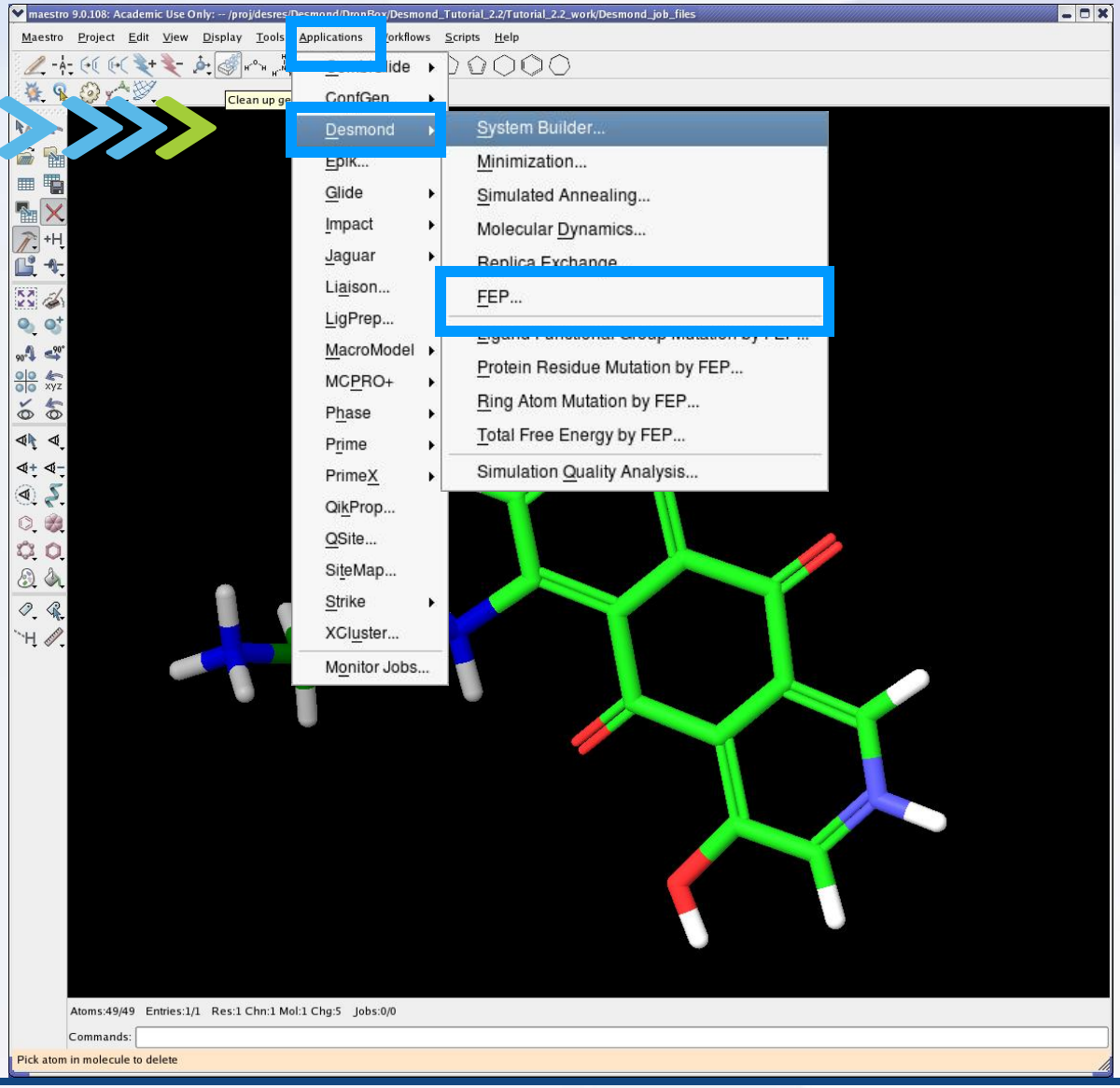
Running free energy perturbation

Three ways of running FEP simulations:

- Write a Desmond configuration file yourself and run the FEP simulation from the command line
- Use Maestro to generate a Desmond configuration file and run the FEP simulation from the command line
- Run the FEP simulation directly from the **FEP Setup** window by clicking **Start**

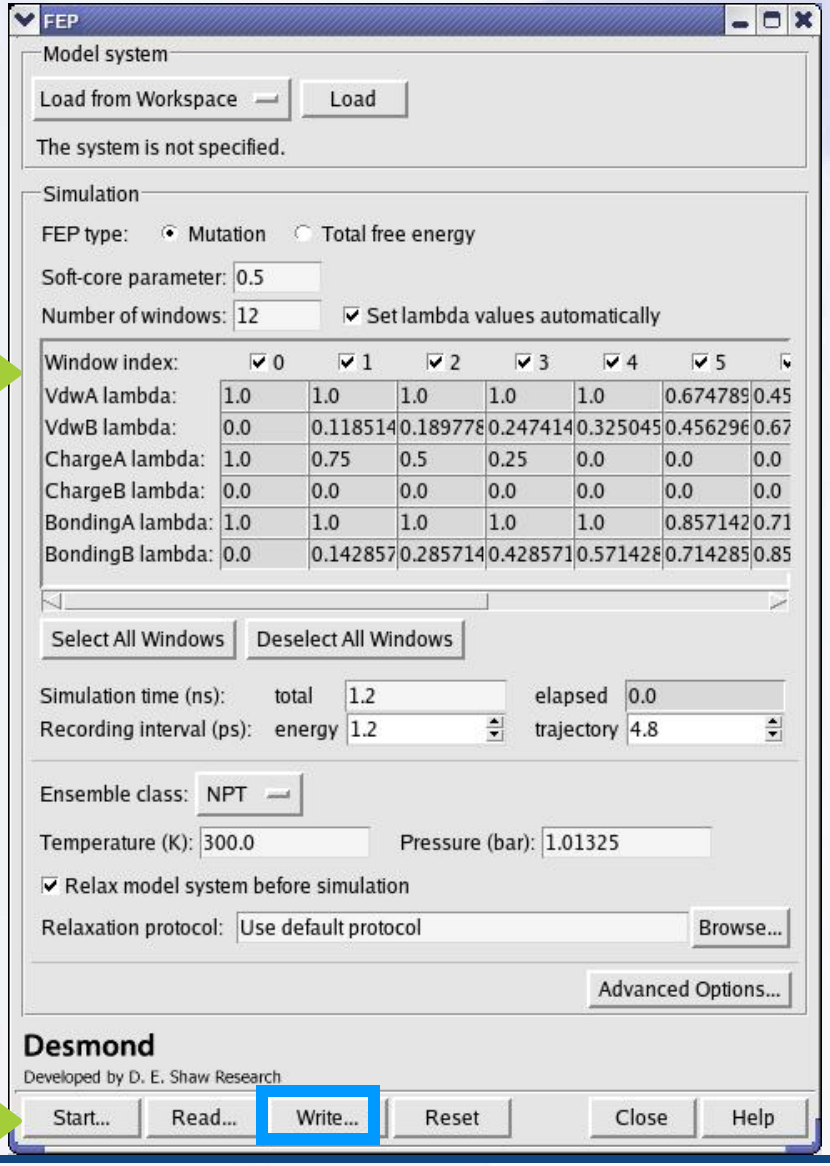
Generating a Desmond FEP configuration file

Select Applications>
Desmond>FEP



Generating a Desmond FEP configuration file

Select the windows that should be included in the simulation


FEP
Model system
Load from Workspace
Load
The system is not specified.
Simulation
FEP type: Mutation Total free energy
Soft-core parameter: 0.5
Number of windows: 12
Set lambda values automatically
Window index: 0 1 2 3 4 5
VdwA lambda: 1.0 1.0 1.0 1.0 1.0 0.674789 0.45
VdwB lambda: 0.0 0.118514 0.189778 0.247414 0.325045 0.456296 0.67
ChargeA lambda: 1.0 0.75 0.5 0.25 0.0 0.0 0.0
ChargeB lambda: 0.0 0.0 0.0 0.0 0.0 0.0 0.0
BondingA lambda: 1.0 1.0 1.0 1.0 1.0 0.857142 0.71
BondingB lambda: 0.0 0.142857 0.285714 0.428571 0.571428 0.714285 0.85
Select All Windows
Deselect All Windows
Simulation time (ns): total 1.2 elapsed 0.0
Recording interval (ps): energy 1.2 trajectory 4.8
Ensemble class: NPT
Temperature (K): 300.0
Pressure (bar): 1.01325
Relax model system before simulation
Relaxation protocol: Use default protocol
Browse...
Advanced Options...
Desmond
Developed by D. E. Shaw Research
Start...
Read...
Write...
Reset
Close
Help

Click **Write**

Running FEP simulation from the command line

- Run FEP simulations using a .msj command script file

```
$SCHRODINGER/utilities/multisim -JOBNAME <jobname>
-maxjob <maxjob> -cpu <cpu> -HOST localhost
-host <hostname> -i <jobname>.mae -m <jobname>.msj
-o <jobname>-out.mae
```

  - `jobname` is the name of the job
  - `maxjob` is the maximum number of subjobs that can be submitted simultaneously (0 means no limit)
  - `cpu` is the number of CPUs per subjob (8 CPUs can be shown as "2 2 2")
  - `-HOST localhost` means the master job runs on your local workstation
  - `-host <hostname>` is the host or queue where the simulation jobs will run
- To apply non-default FEP parameters or change the number of Lambda windows, add the `-cfg <config_file>` option and provide the name of the file generated by the FEP window

Computing free energy difference

- Run the bennett.py script from the command line to process the "energy" file output of the different Lambda runs and compute the free energy

```
$SCHRODINGER/run -FROM desmond bennett.py [--help]
```

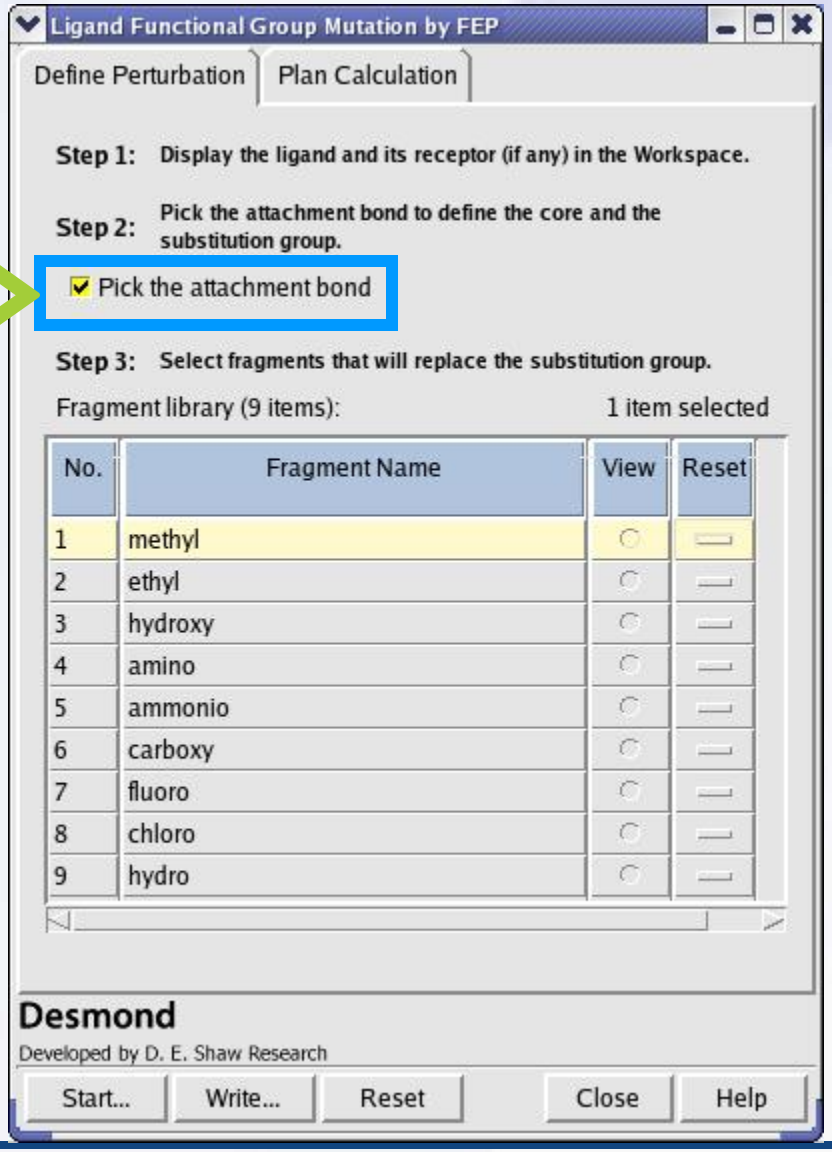
- or from Maestro, import the –**out.cms** file and the delta G free energy difference can be displayed in the Project Table
- The delta-delta G relative binding free energy difference is equal to delta G "complex" – delta G "solvent"

Creating a custom fragment group

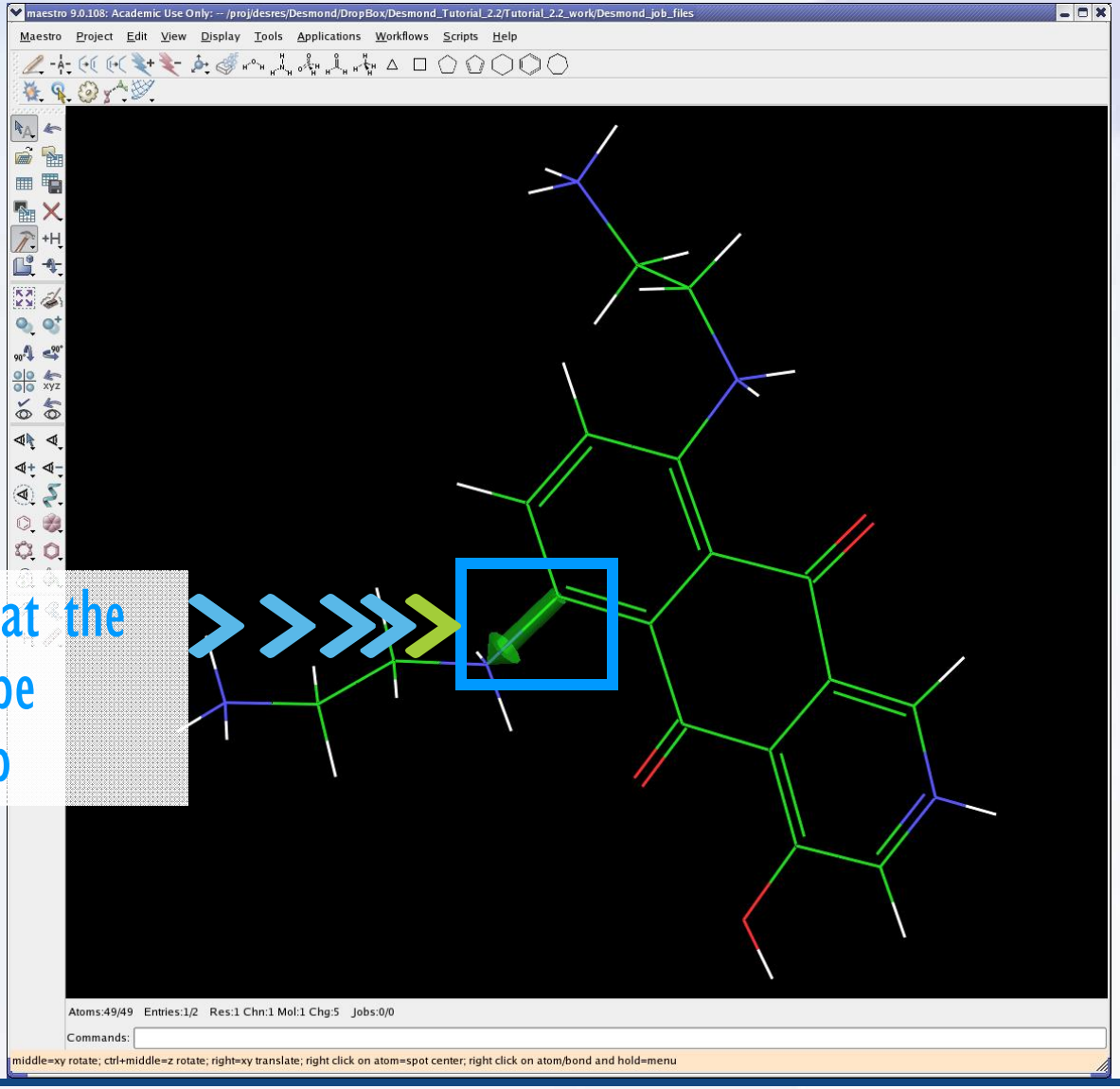
- Edit any pre-defined substitution group manually with the **Build/Edit** tools in Maestro
- Mutations of more than a few atoms are subject to large uncertainties in the free energy differences computed between different Lambda windows
- Decompose large mutations into several smaller mutations and run multiple MultiSim FEP simulations

Creating a custom fragment group

Select the **Pick the attachment bond** option



Creating a custom fragment group

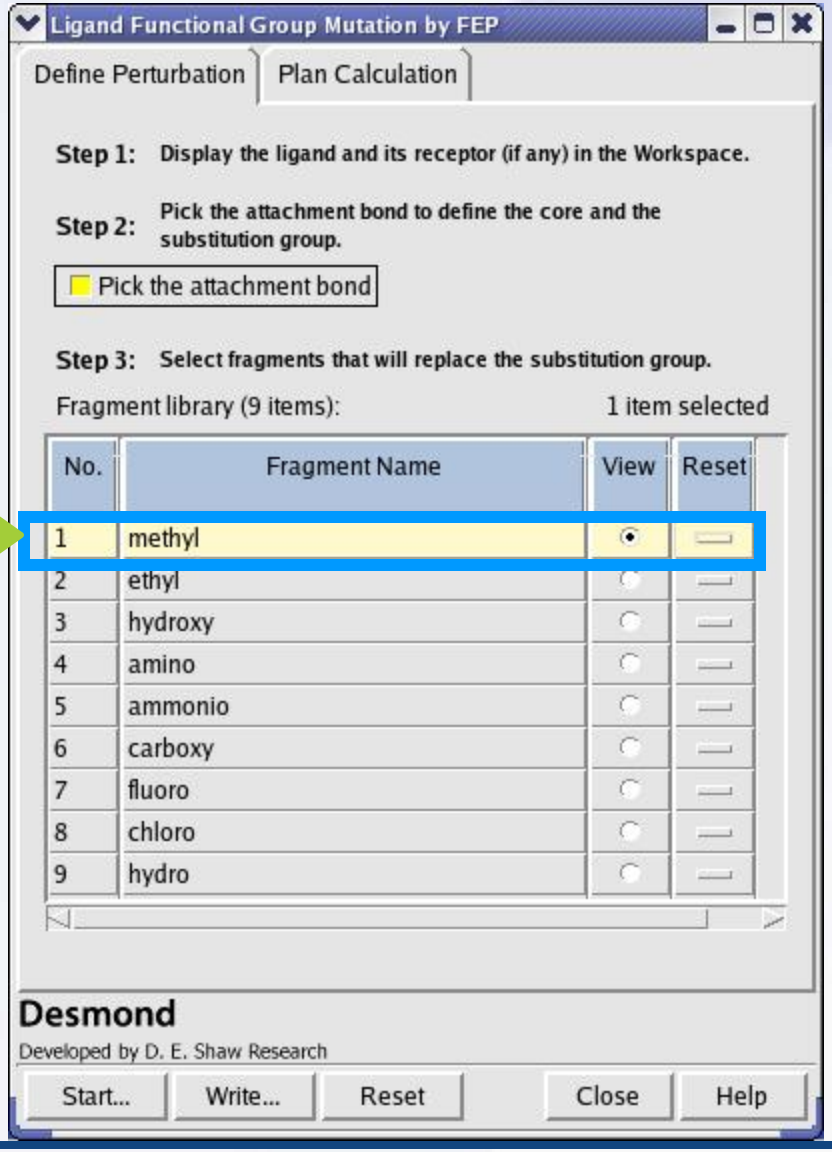

maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:49/49 Entries:1/2 Res:1 Chn:1 Mol:1 Chg:5 Jobs:0/0
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Pick the attachment bond at the base of the side chain to be mutated into a butyl group

Creating a custom fragment group

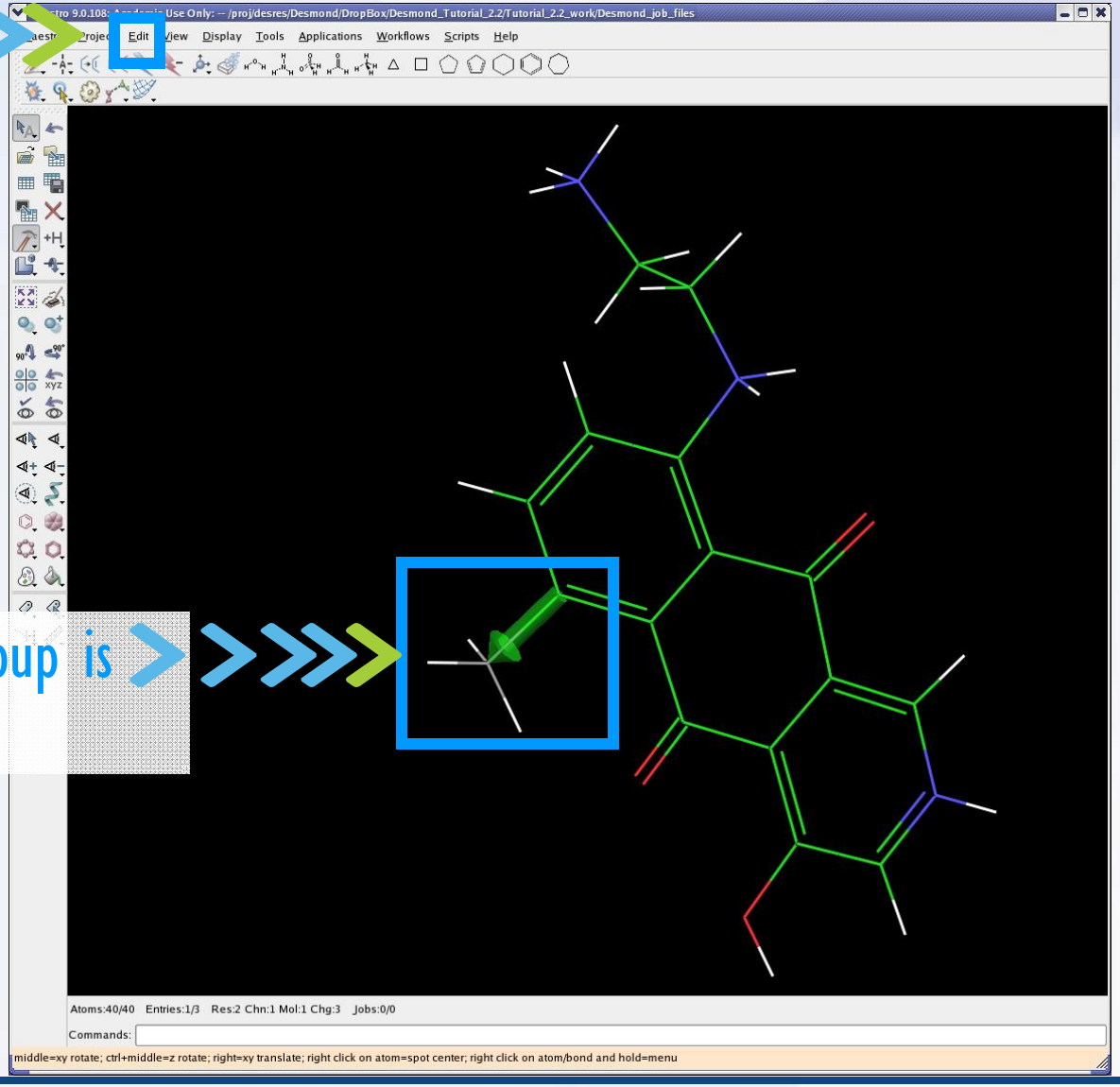
Select the methyl fragment as the base of the butyl substitution group

Click **View**



Creating a custom fragment group

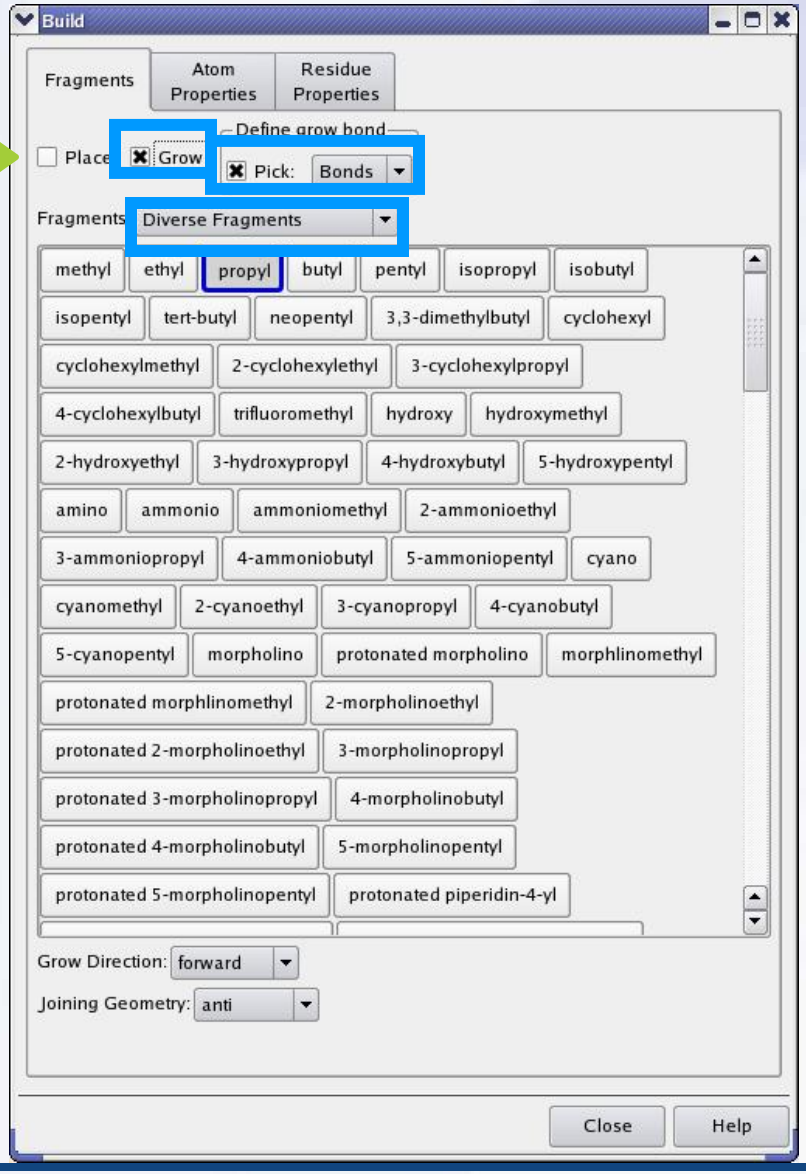
Select Edit> Fragments



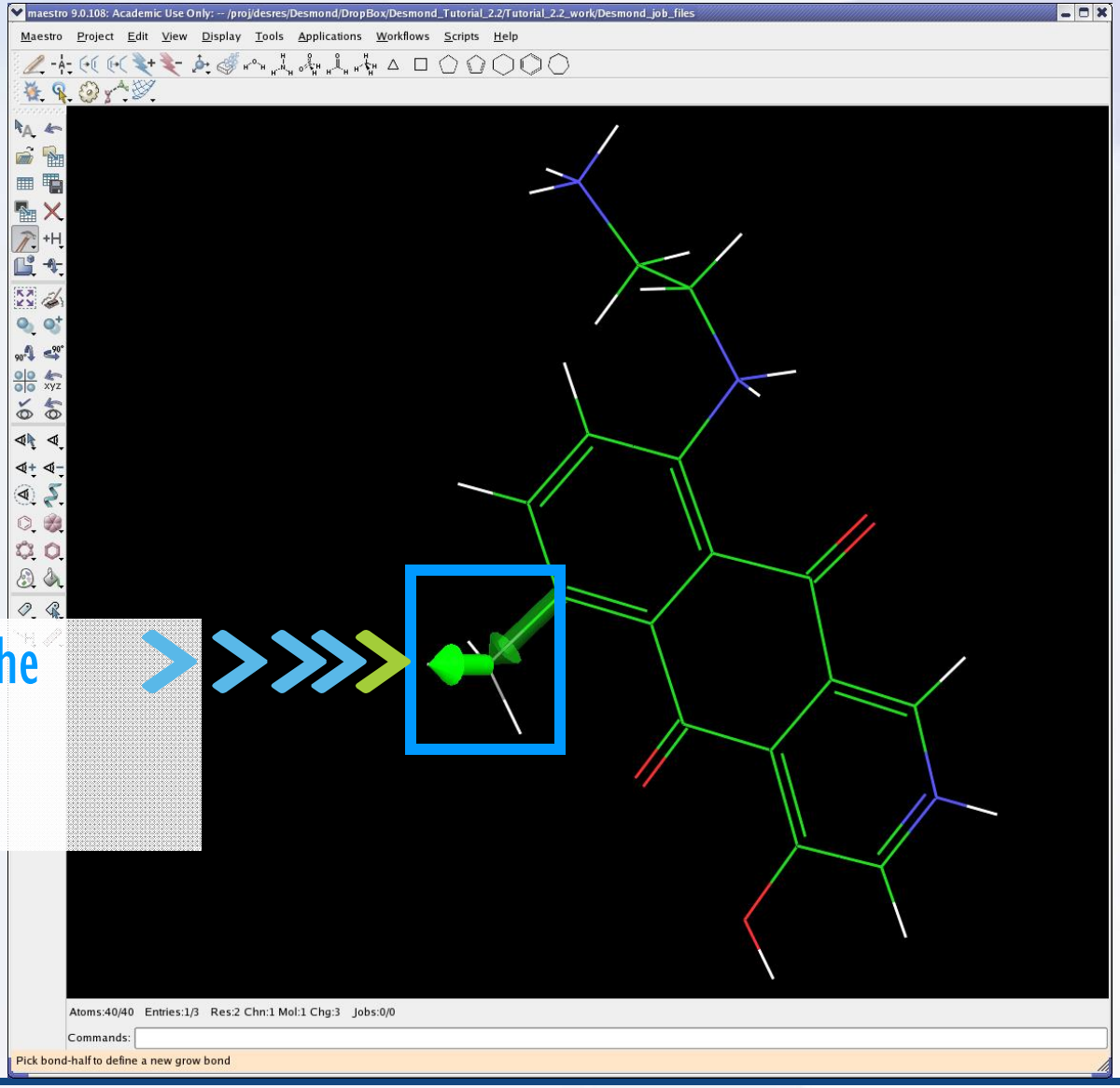
The methyl substitution group is shown in the Workspace

Creating a custom fragment group

Select the **Grow** option
Select the **Pick** option and choose **Bonds**
Select **Diverse Fragments** from **Fragments**



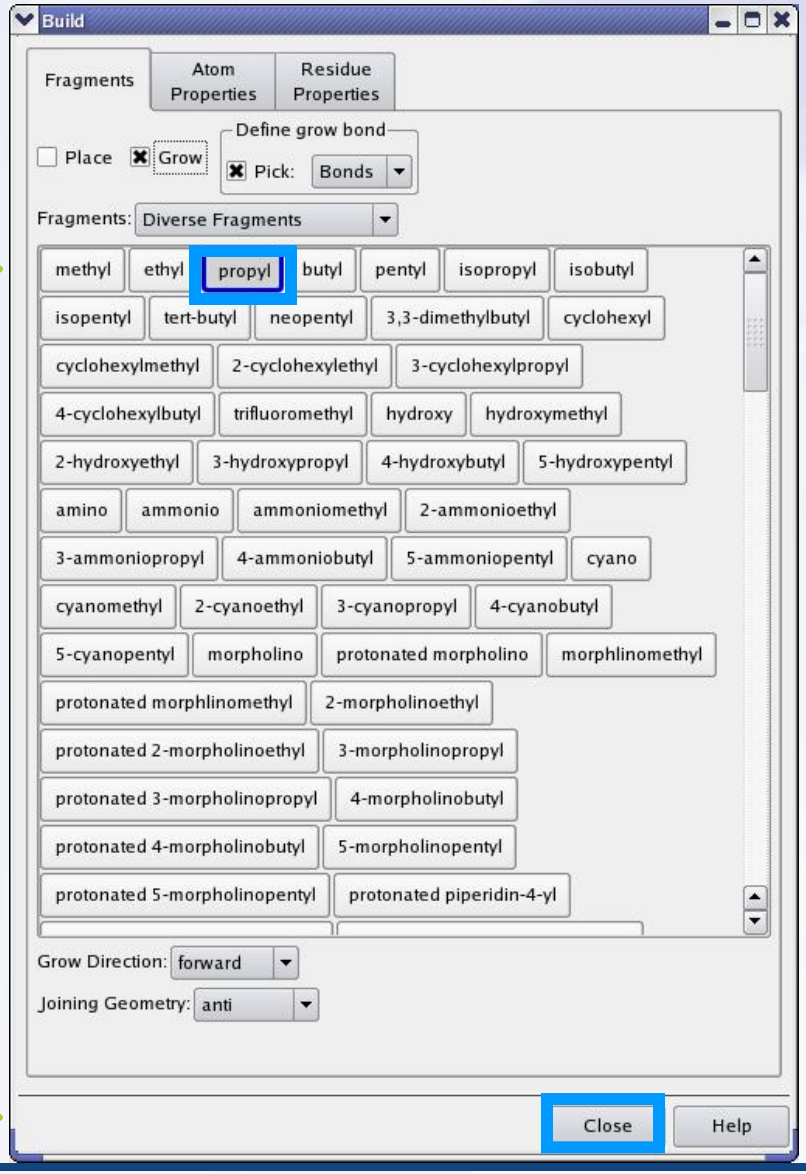
Creating a custom fragment group


maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:40/40 Entries:1/3 Res:2 Chn:1 Mol:1 Chg:3 Jobs:0/0
Commands:
Pick bond-half to define a new grow bond

Select the grow bond in the Workspace
(the bright green arrow)

Creating a custom fragment group

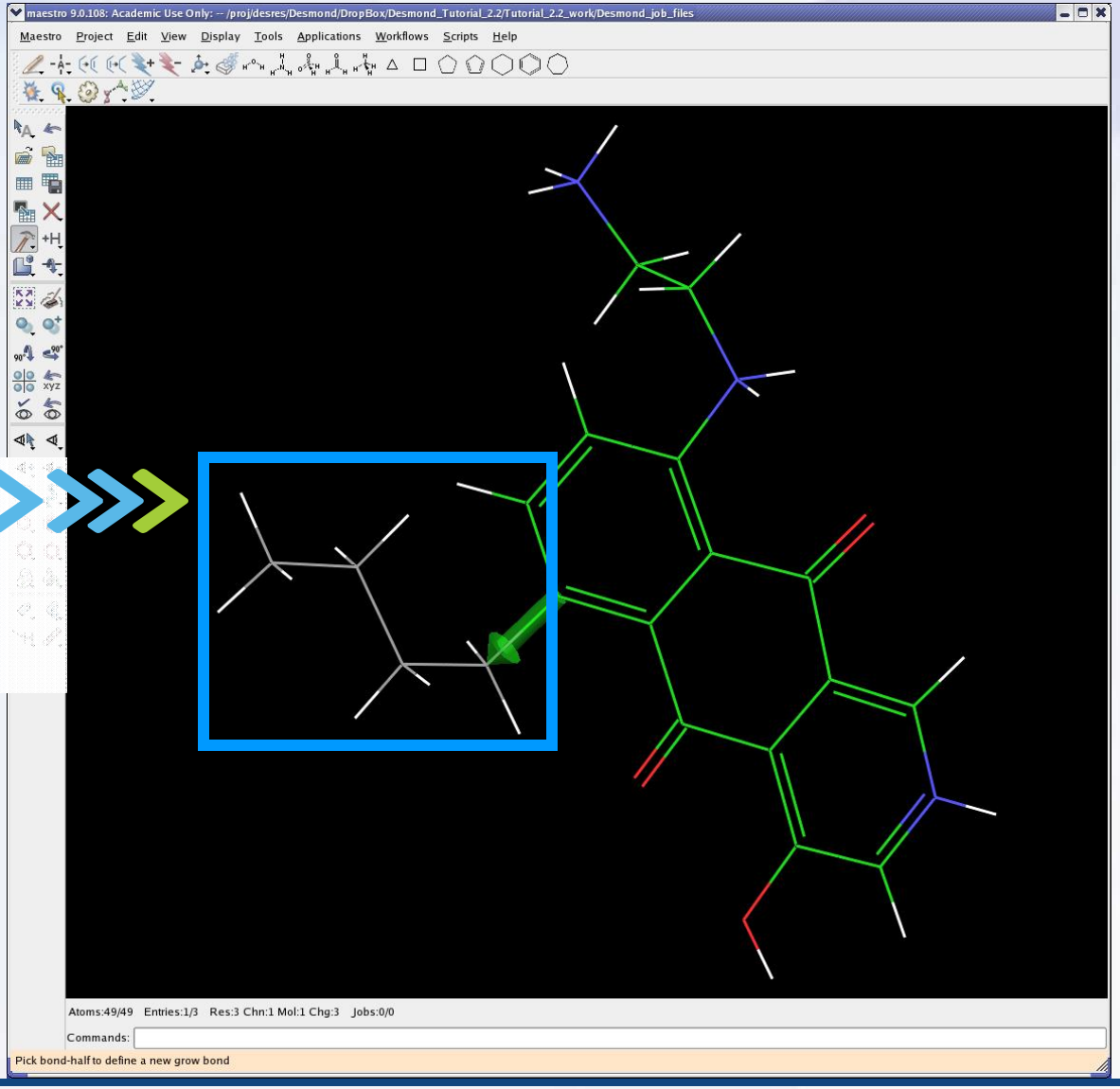
Select propyl under Fragments



Click Close

Creating a custom fragment group

The butyl substitution group is shown in the Workspace


maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:49/49 Entries:1/3 Res:3 Chn:1 Mol:1 Chg:3 Jobs:0/0
Commands:
Pick bond-half to define a new grow bond

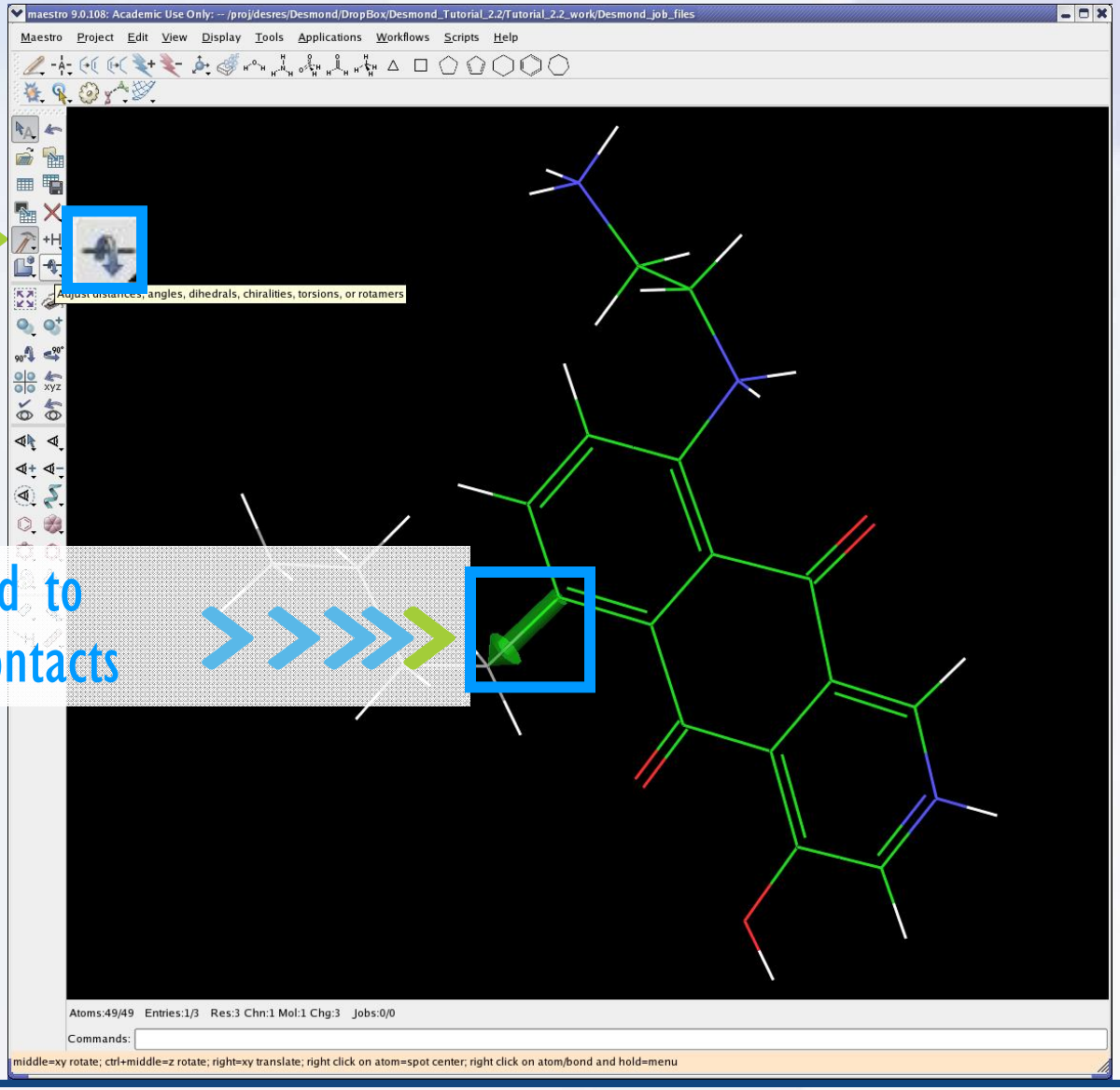
Adjusting the conformation of the mutant

- Initial conformation of the mutant must be as close as possible to the original side chain for FEP simulations
- Manual adjustments to the conformation of small molecules can be readily done in Maestro
- Use Maestro's Adjustment tool to make manual adjustments

Adjusting the conformation of the mutant

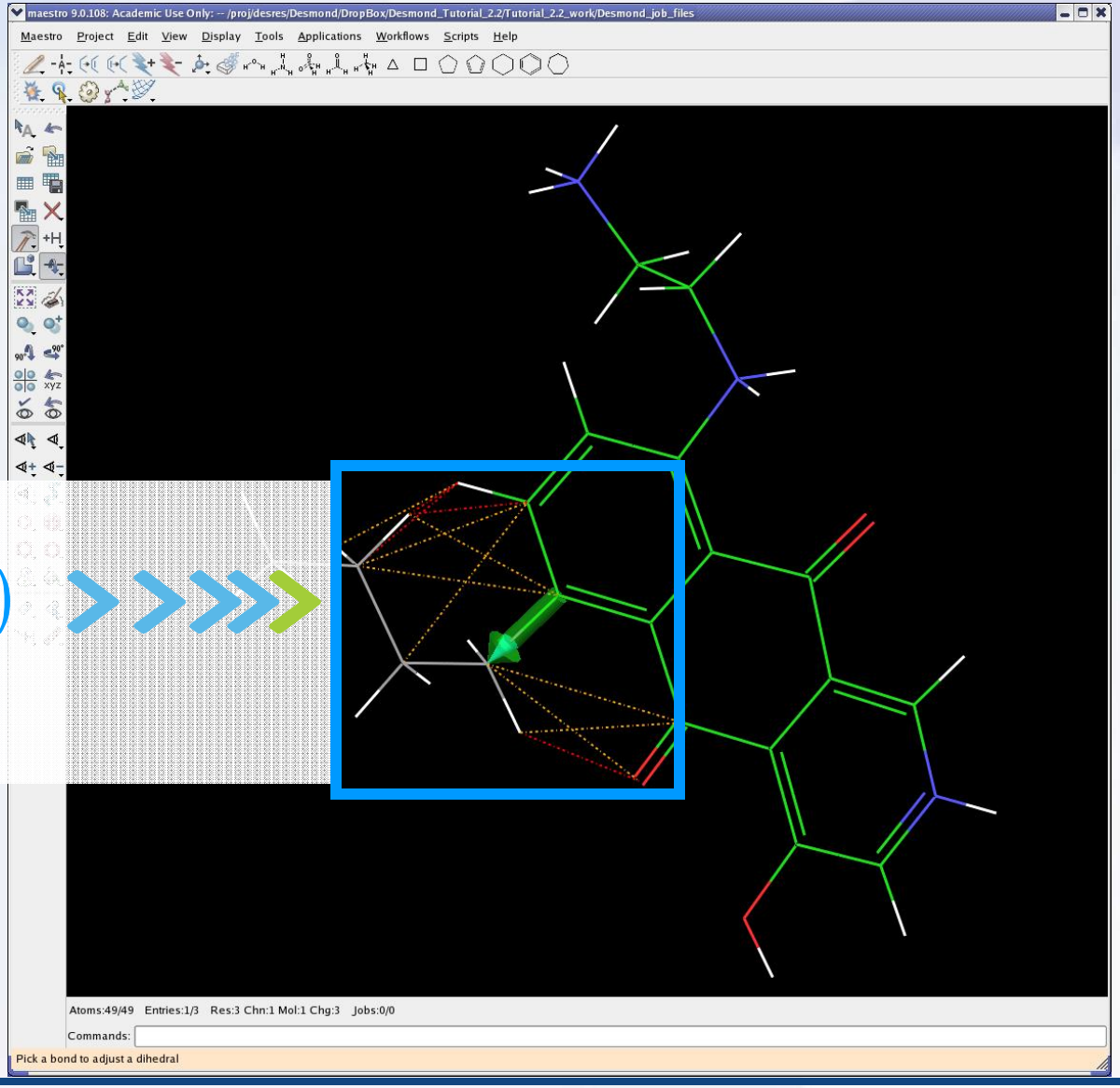
Click the **Adjustment** icon to make manual adjustments

Select the attachment bond to display the non-bonded contacts


maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:49/49 Entries:1/3 Res:3 Chn:1 Mol:1 Chg:3 Jobs:0/0
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Adjusting the conformation of the mutant

Note the numerous close contacts (shown in orange) and a few unfavorable contacts (shown in red)


maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:49/49 Entries:1/3 Res:3 Chn:1 Mol:1 Chg:3 Jobs:0/0
Commands:
Pick a bond to adjust a dihedral

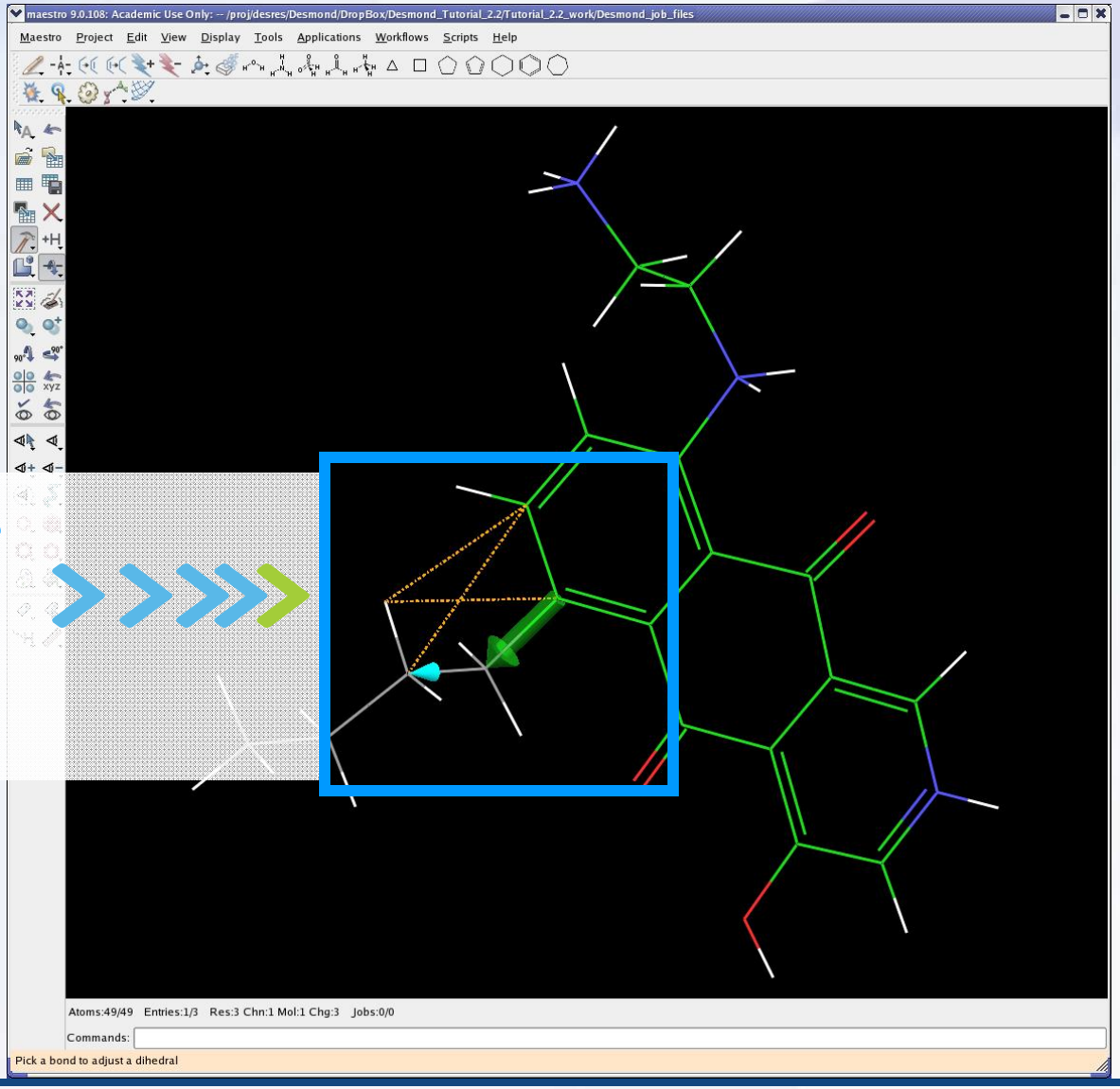
Adjusting the conformation of the mutant

- Adjust the torsion angle manually using the **Adjustment Tool**'s "Quick Torsion" option:
  - Hold down the left mouse button and move the mouse horizontally
  - or Rotate the mouse wheel
- Relax the unfavorable contacts iteratively with rotations about different bonds in the side chain

Adjusting the conformation of the mutant

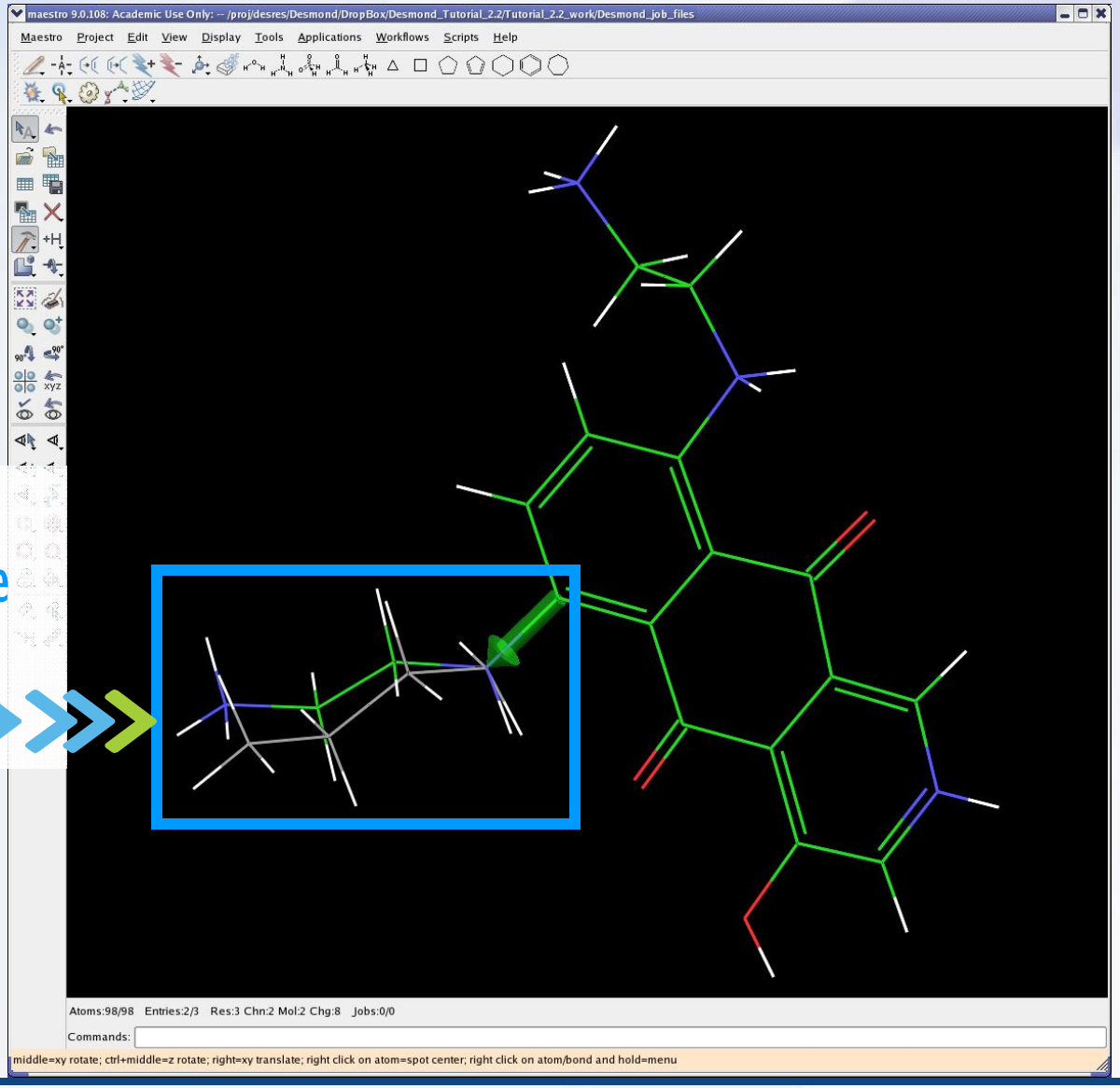
Only allowed close contacts remain

Unfavorable contacts have been removed


maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:49/49 Entries:1/3 Res:3 Chn:1 Mol:1 Chg:3 Jobs:0/0
Commands:
Pick a bond to adjust a dihedral

Adjusting the conformation of the mutant

The original side chain (shown in green) alongside the modified side chain (shown in grey)


maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
Atoms:98/98 Entries:2/3 Res:3 Chn:2 Mol:2 Chg:8 Jobs:0/0
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Other types of mutations

- Protein residue mutation
  - Compute the difference in free energy for a ligand bound to a protein versus the same ligand bound to a mutated form of the same receptor with amino acid residue mutations applied to the protein
  - Compute the difference in free energy for a protein versus a mutated protein, without a ligand
- Ring atom mutation — replace one atom with another in a ring structure
- Absolute or total free energy calculation
  - Compute absolute solvation free energy (and absolute binding free energy) by annihilating a whole solute or ligand molecule

Visualization and analysis using Maestro

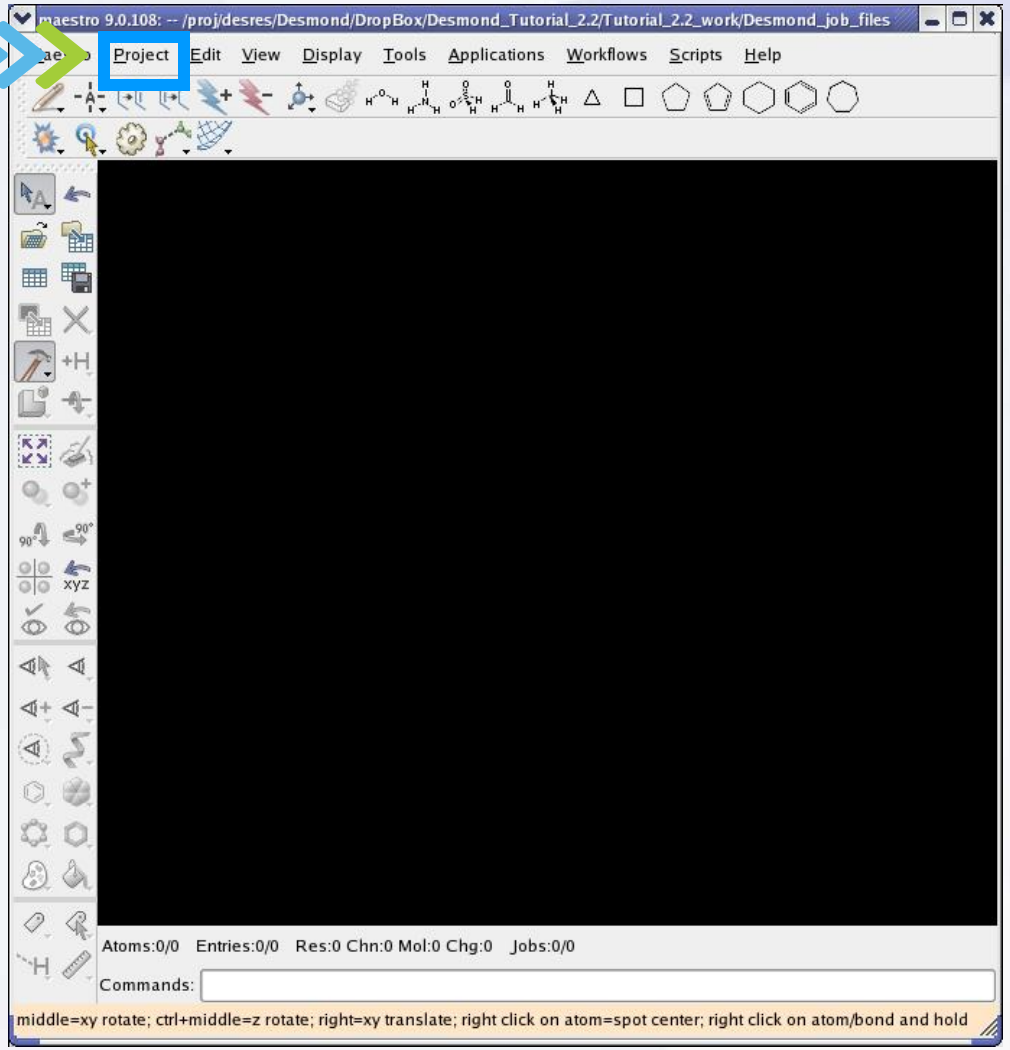
- Maestro offers basic visualization and analysis tools
- Trajectory Player
  - Similar to the ePlayer in the Project Table
  - Designed to animate Desmond trajectories
- Simulation Quality Analysis window
  - Performs basic quality analysis
  - Shows statistical properties of basic thermodynamic quantities based on block averages
- Schrödinger add-on scripts
  - Simulation Event Analysis
  - Trajectory cluster analysis

Visualization and analysis using Maestro

Trajectory Player

Select **Project>Import Structures**

In the **Import** panel, select **CMS** from the **Files of type** field, and select one of the ***-out.cms** files

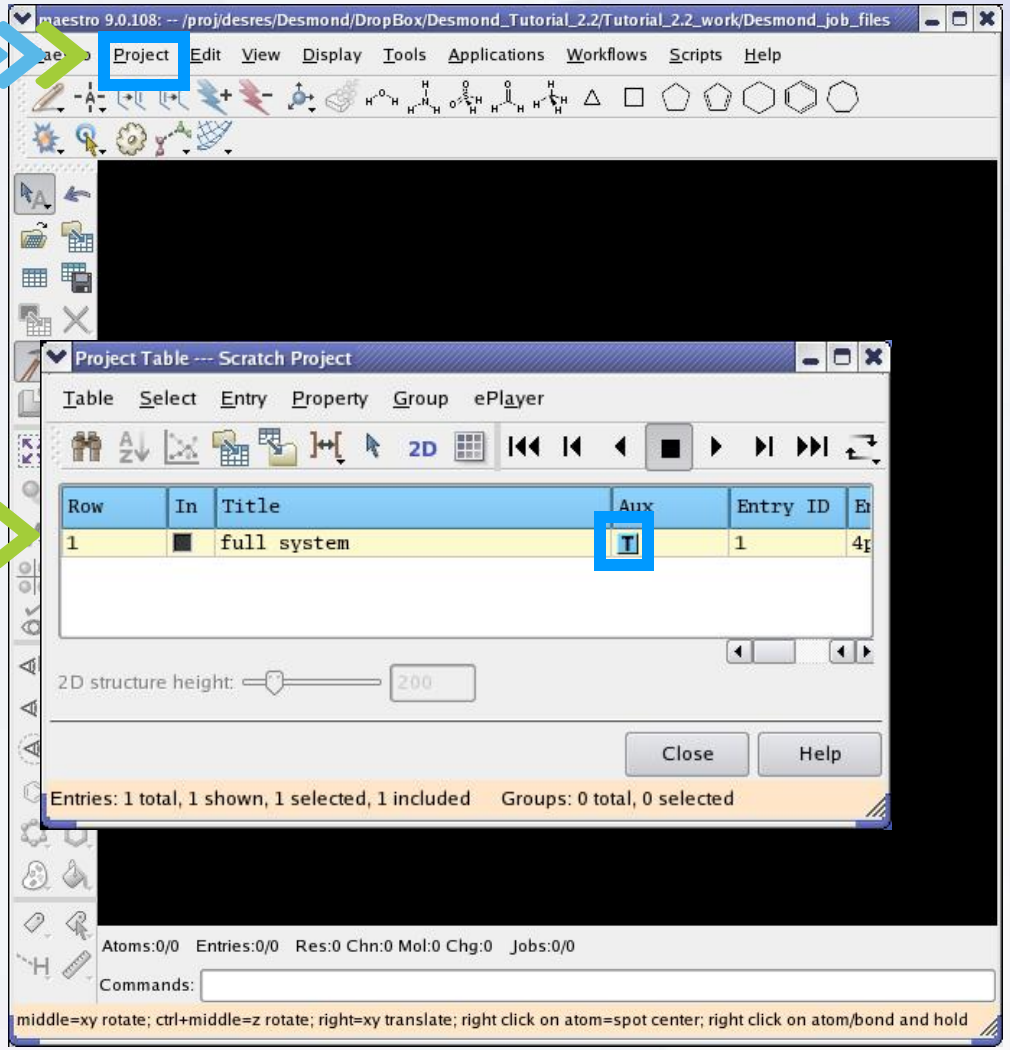


Visualization and analysis using Maestro

Trajectory Player

Select **Project>Show Table** >>>>

In the **Project Table** click the **T** button in the **Aux** column >>>>



Visualization and analysis using Maestro

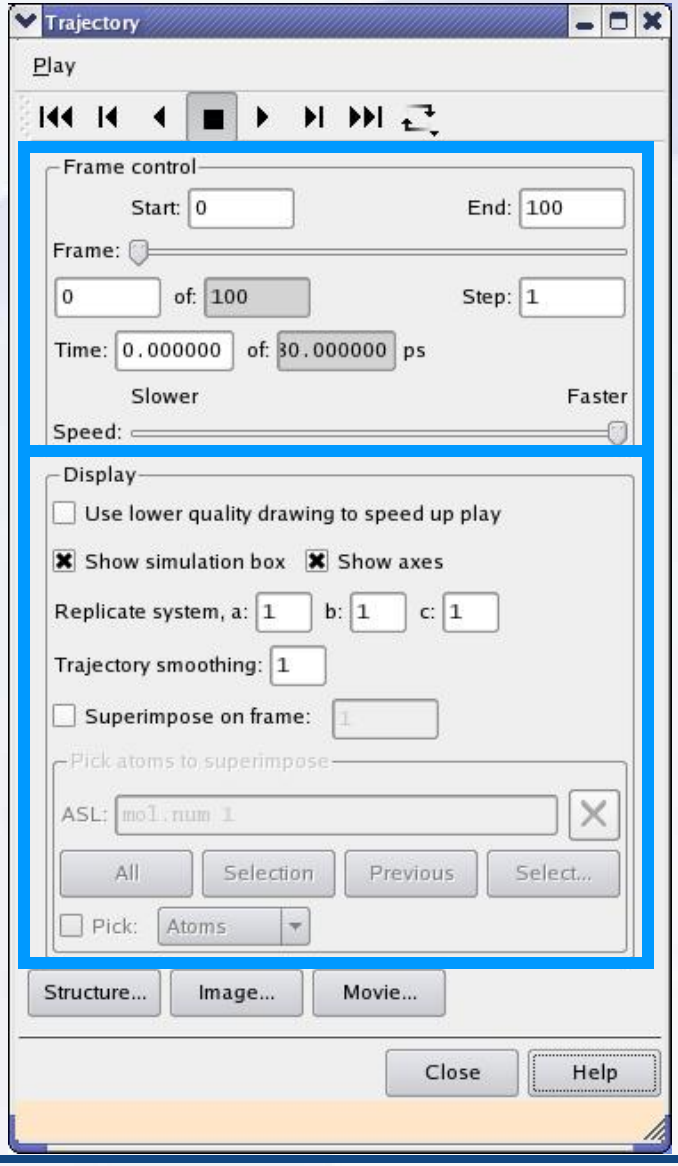
Trajectory Player

Options for frame control

Options for display “look and feel”

The Trajectory Player will generate a smooth animation that you can save as a MPEG movie

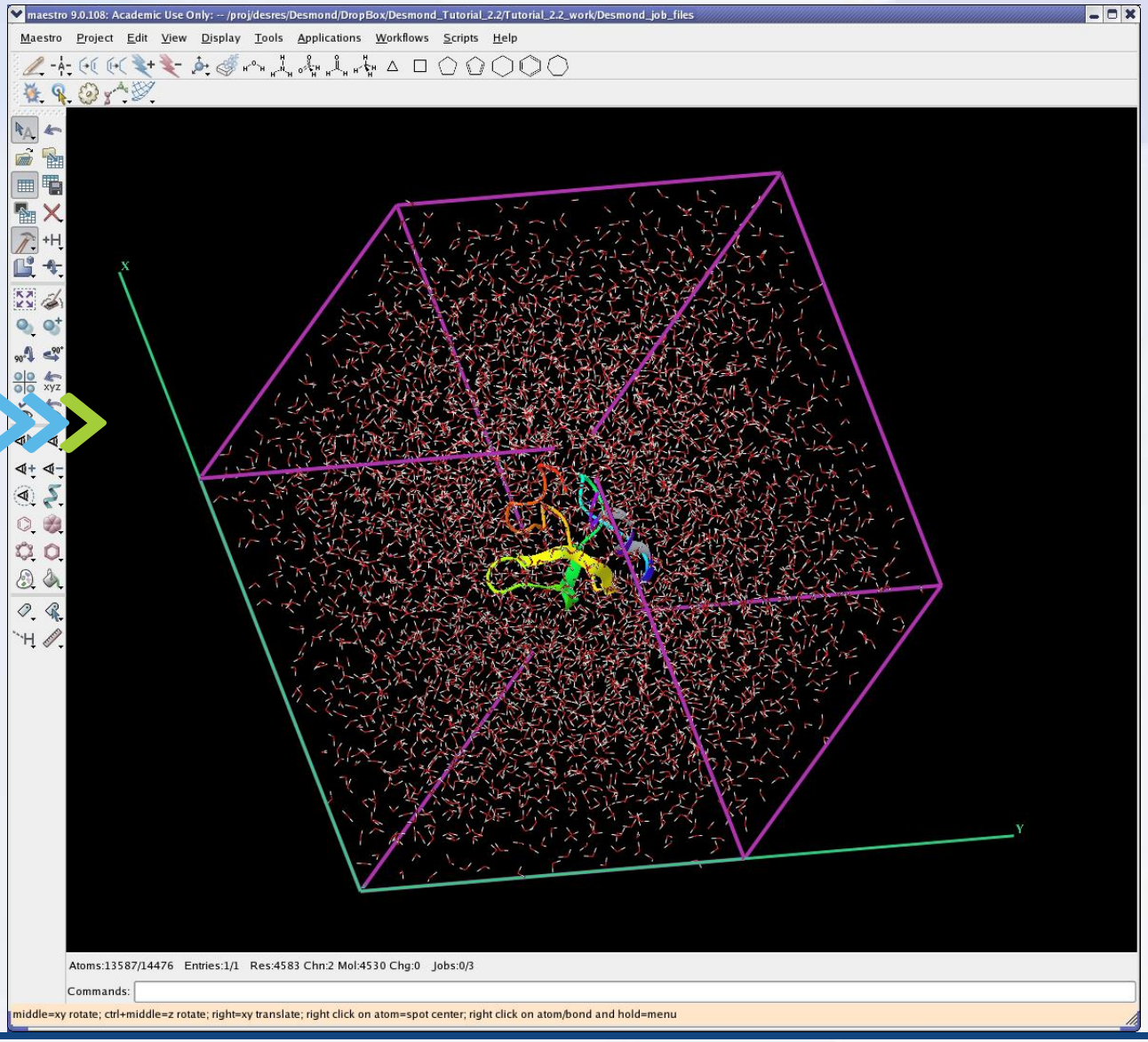
You can take snapshots of the trajectory and save it in Maestro format



Visualization and analysis using Maestro

Trajectory Player

Workspace view for trajectory visualization


maestro 9.0.108: Academic Use Only: -- /proj/desres/Desmond/DropBox/Desmond_Tutorial_2.2/Tutorial_2.2_work/Desmond_job_files
Maestro Project Edit View Display Tools Applications Workflows Scripts Help
X
Y
Atoms:13587/14476 Entries:1/1 Res:4583 Chn:2 Mol:4530 Chg:0 Jobs:0/3
Commands:
middle=xy rotate; ctrl+middle=z rotate; right=xy translate; right click on atom=spot center; right click on atom/bond and hold=menu

Visualization and analysis using Maestro

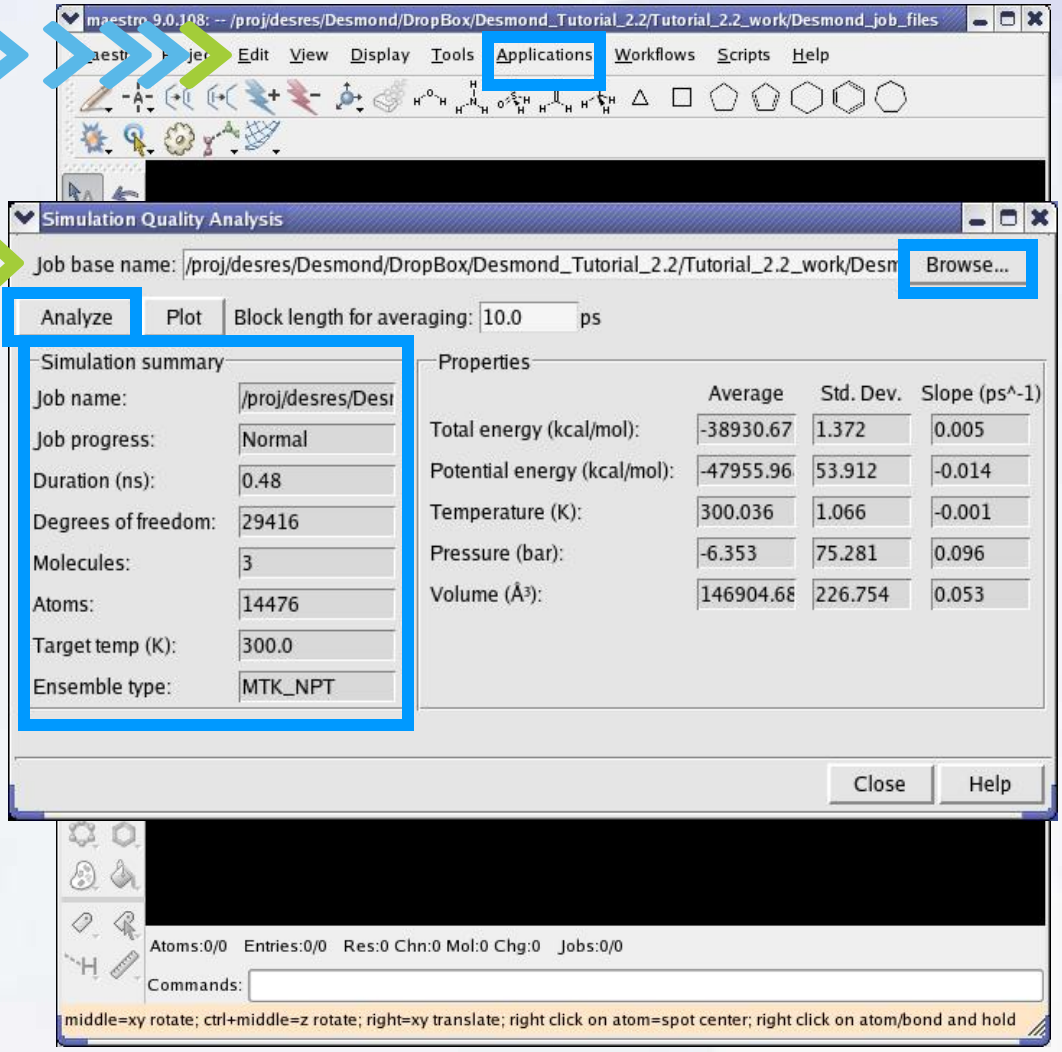
Simulation Quality Analysis

Select **Applications > Desmond > Simulation Quality Analysis**

Click **Browse** and select the energy file (**desmond_job.ene**)

Click the **Analyze** button

Results of analysis appear

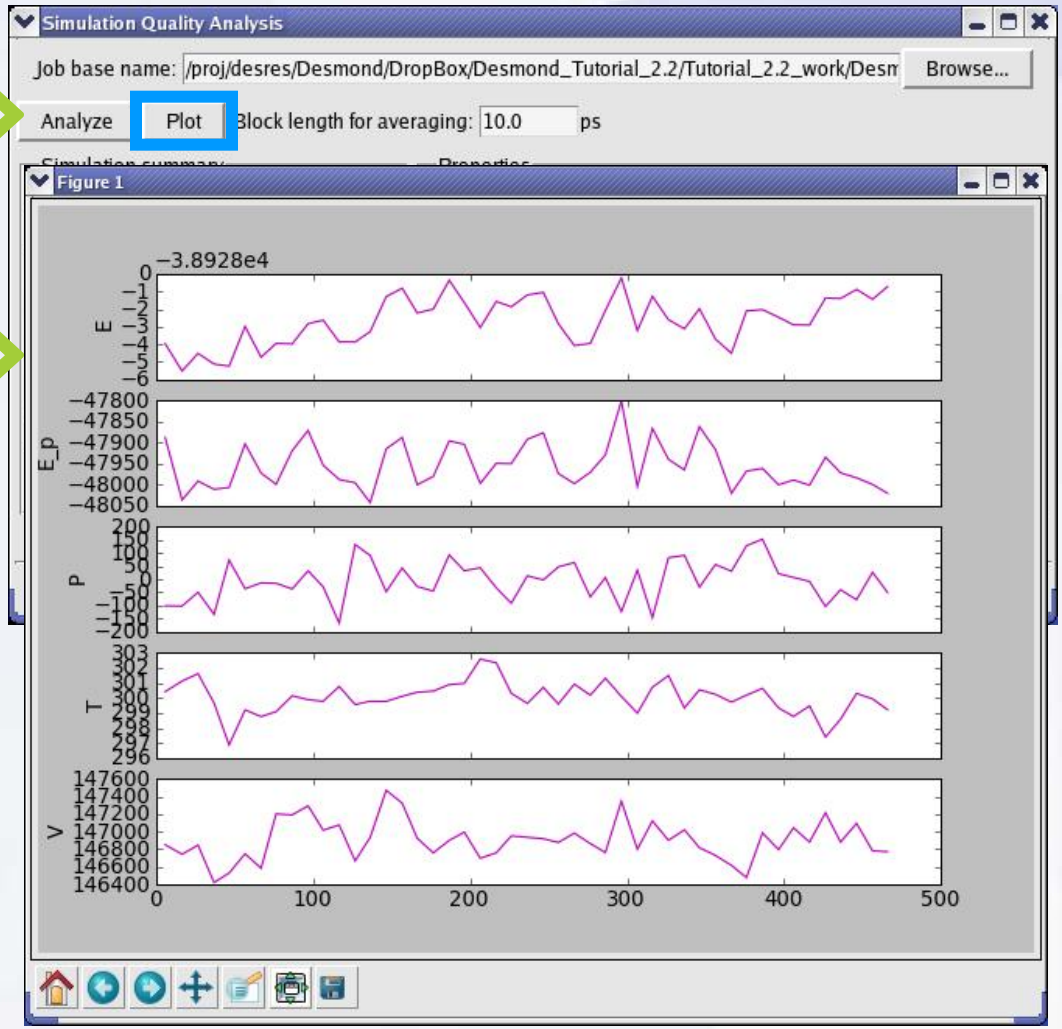


Visualization and analysis using Maestro

Simulation Quality Analysis

Click the **Plot** button

An interactive graphical representation of the data appears



Trajectory analysis with VMD

- VMD is a widely used molecular visualization platform providing a plethora of tools for trajectory analysis:
  - Strong support for atom selections
  - Ability to efficiently animate trajectories with thousands of snapshots
  - Support for Tcl and Python scripting languages
  - Built-in capability to write Maestro files (useful to prepare simulation system in VMD) and read Desmond trajectory files (useful for analyzing Desmond trajectories)
    These features are available in the new VMD 1.8.7

Trajectory analysis with VMD

- VMD Python interface
  - Access from console (terminal from which you launched VMD)
    `vmd>gopython`
  - Access from **Idle** window that runs inside VMD (preferred)
    `vmd>gopython -command "import vmdidle; vmidle.start()"`
  - Copy command into **.vmdrc** file
  - Some modules are provided by the Python interpreter when you launch Python, others are loaded at runtime
  - VMD provides built-in modules for loading data into VMD, querying and modifying VMD state: **`molecule`** module, **`atomsel`** module, **`vmdnumpy`** module

Trajectory analysis with VMD

- Loading and viewing trajectories
  - Structure files (.mae or .cms)
    - Created by Maestro or Desmond
    - Contain topology, mass, charge, and possibly force field information
    - Single molecular system including solvent or ions
    - Single snapshot of positions of atoms and virtual sites
    - Select the Maestro **mae** file type to select these files
  - Trajectory files (.dtr)
    - Created by Desmond
    - Directory that contains metadata files and frame files holding snapshots
    - **.dtr** files load positions, **.dtrv** files load positions and velocities
    - Select the **clickme.dtr** file

Trajectory analysis with VMD

- Loading files from the command line
  - **`vmd –mae structure.cms –dtr trajectory_subdir/clickme.dtr`**
  - This command:
    - Creates a new molecule
    - Initializes its structure from the **cms** file
    - Creates an initial set of coordinates from the **cms** file
    - Appends all snapshots from the **dtr** file to the molecule
  - Load multiple structure and trajectory files with -m and -f
    - -m causes a new molecule to be created for each subsequent file
    - -f causes subsequent files to be loaded into the same molecule
    - **`vmd -m -mae mol1.mae mol2.mae -f mol3.mae -dtr trajectory_subdir/clickme.dtr`**

Trajectory analysis with VMD

Loading files from the VMD window

Select **File>New Molecule**

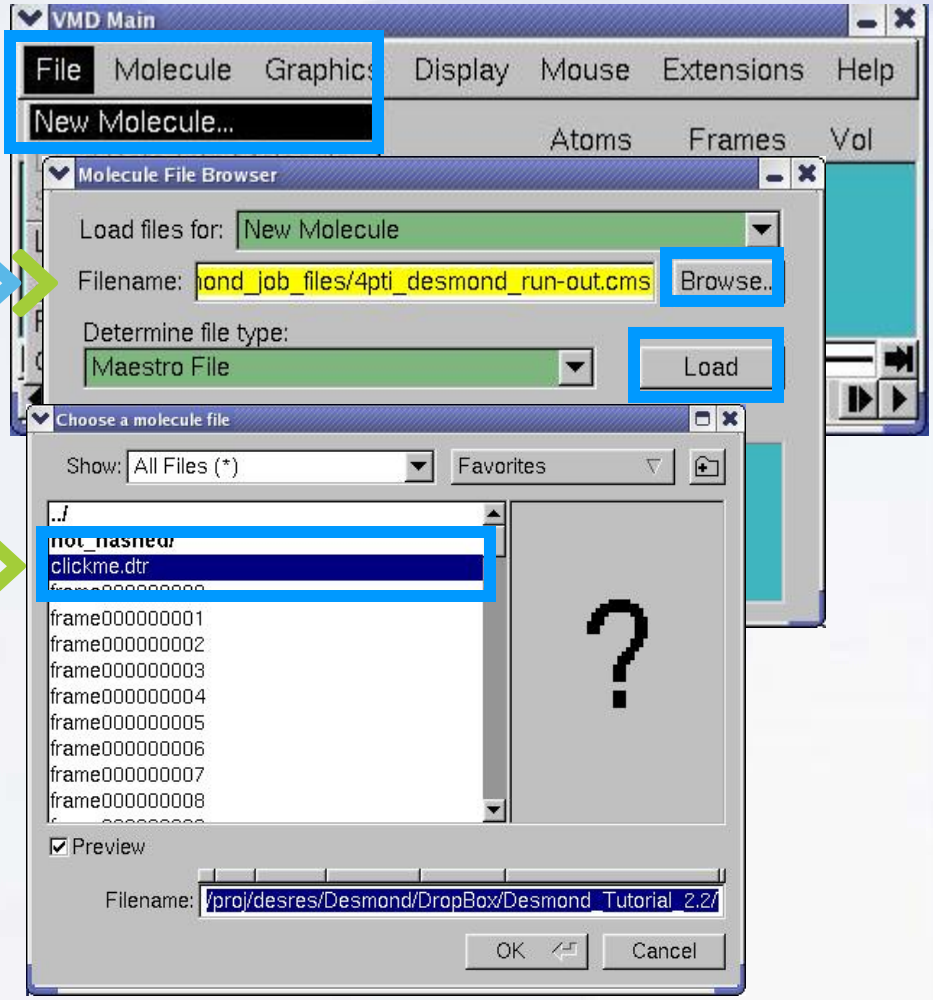
Click **Browse** and select the structure file

Click **Load**

Click **Browse** again

In the **Choose a molecule file** window select the **`clickme.dtr`** "trajectory file" you want to use

Click **Load** again

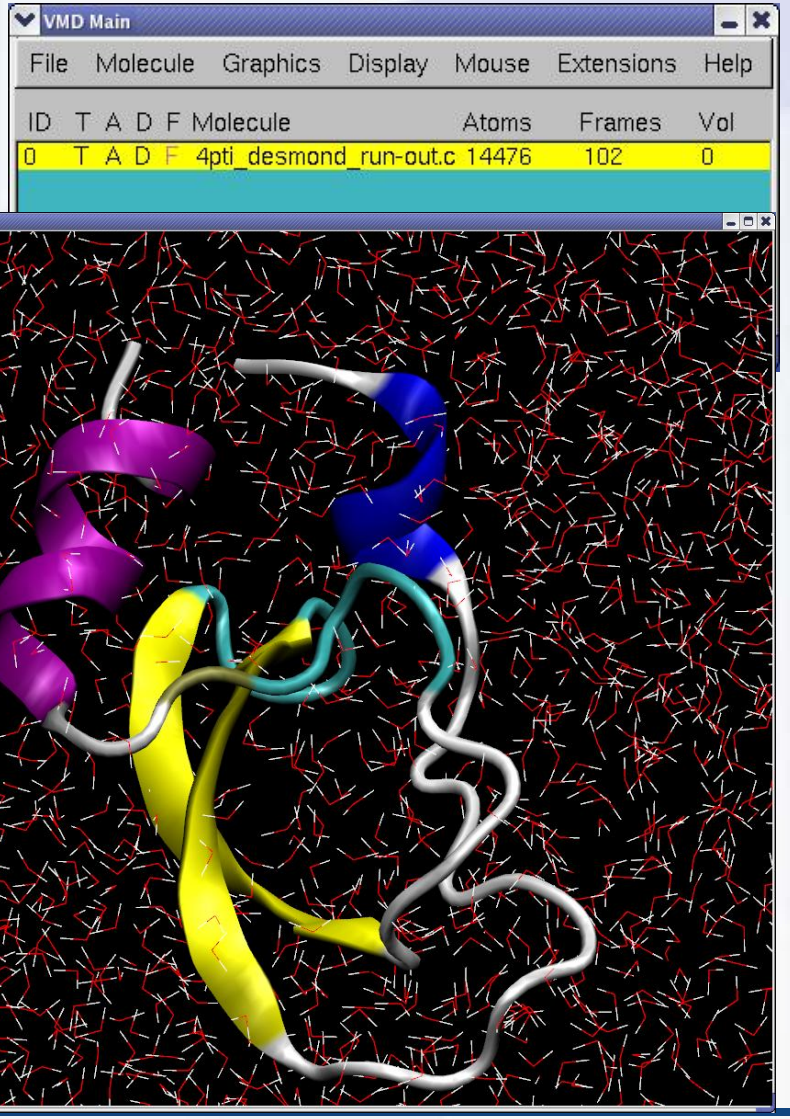


Trajectory analysis with VMD

Loading files from the VMD window

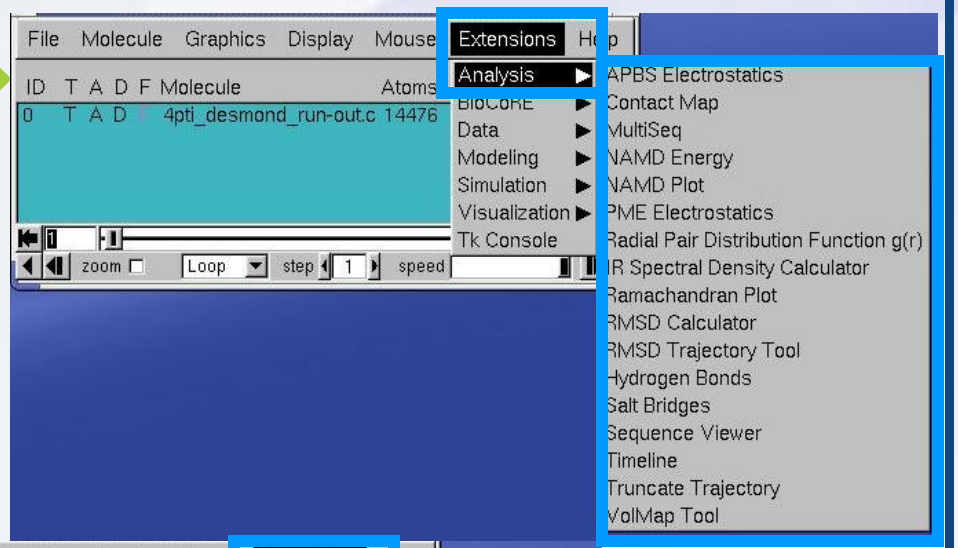
Molecular information appears in the **VMD Main** window

Trajectory movie appears in the **VMD OpenGL Display** window

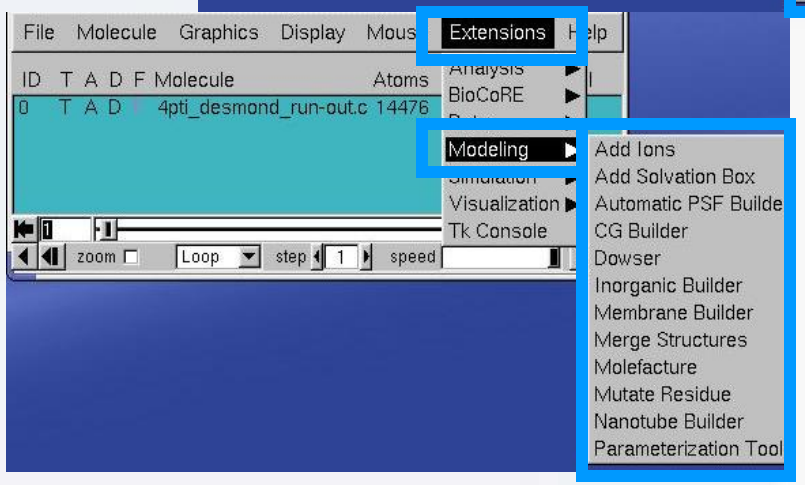

VMD Main
File Molecule Graphics Display Mouse Extensions Help
ID T A D F Molecule Atoms Frames Vol
0 T A D F 4pti_desmond_run-out.c 14476 102 0
VMD 1.8.7a58 OpenGL Display

Trajectory analysis with VMD

Select **Extensions>Analysis** for trajectory analysis tools



Select **Extensions >Modeling** to set up simulation systems in VMD



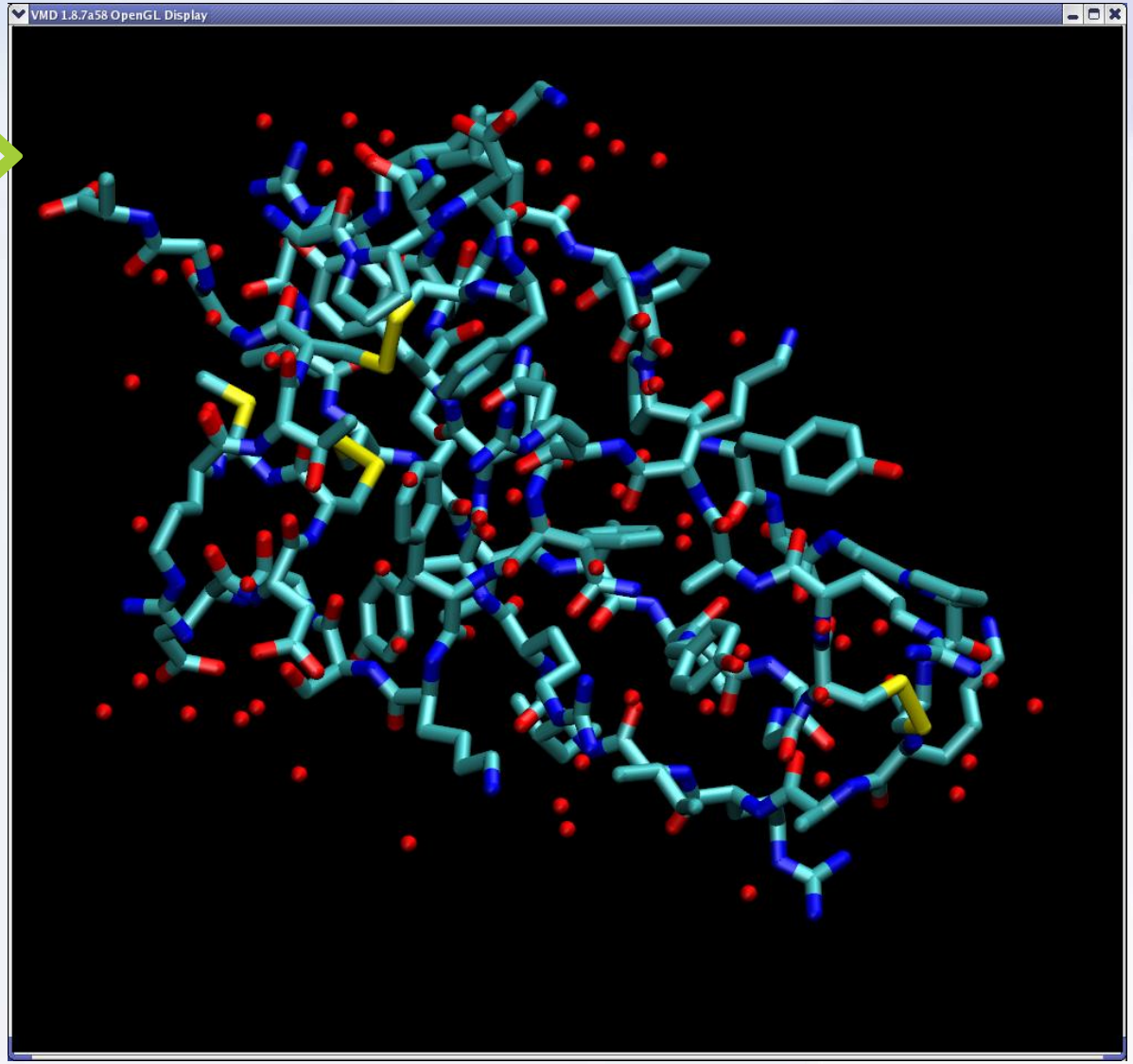
Preparing molecular systems with VMD

- Load a molecule into VMD
- Use VMD modeling tools to generate the simulation system (the main building tool is Automatic PSF Builder)
- Save the system in a Maestro file (**File>Save Coordinates**)
- Add force field parameters and constraints with *Viparr* and the **build_constraints** script

Preparing molecular systems with VMD

Load the protein structure (PDB file) into the **VMD Display** window

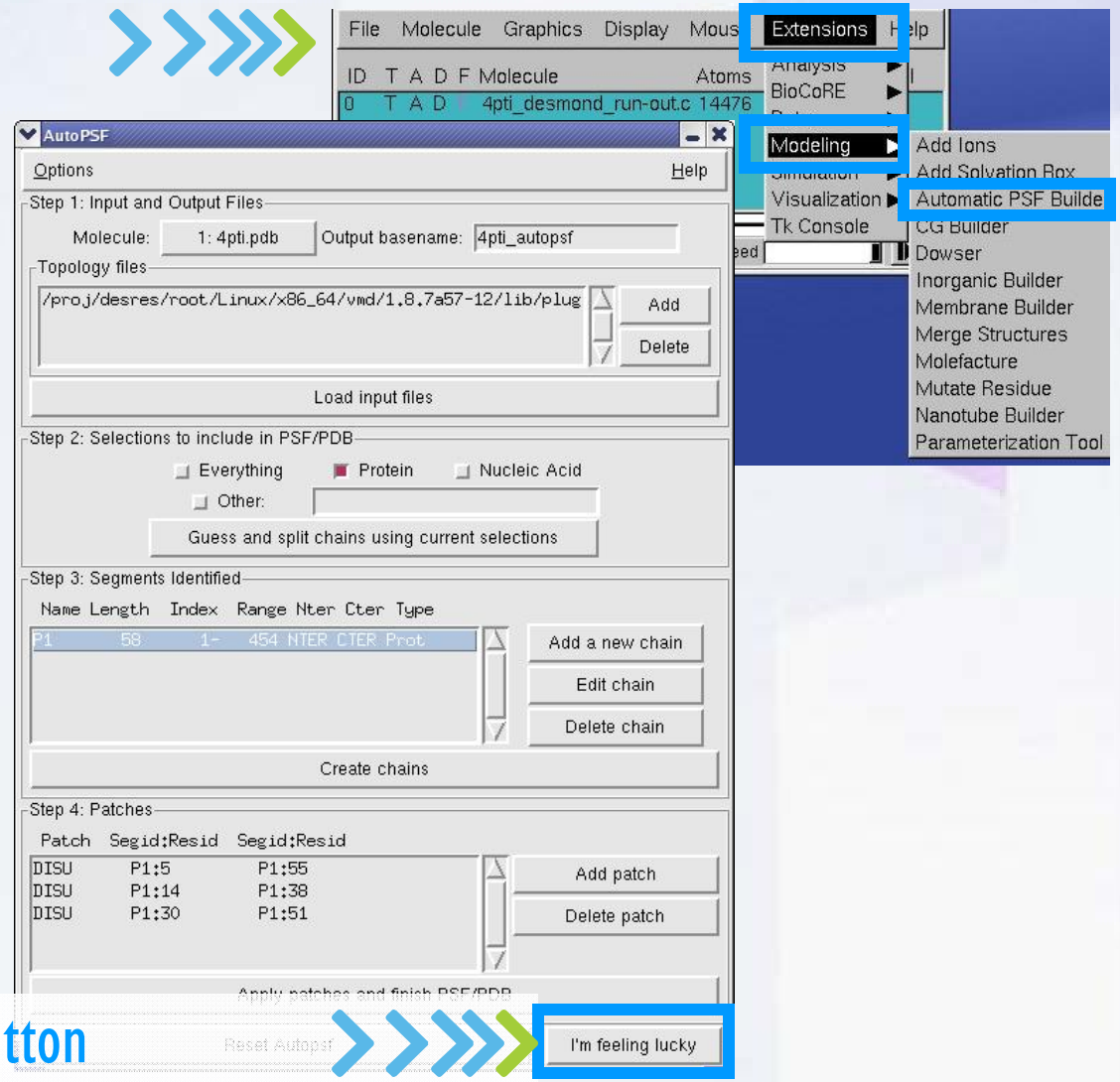
Change the display from wire frame to "licorice" in the **Graphical Representations** window



Preparing molecular systems with VMD

Select Extensions>Modeling> Automatic PSF Builder

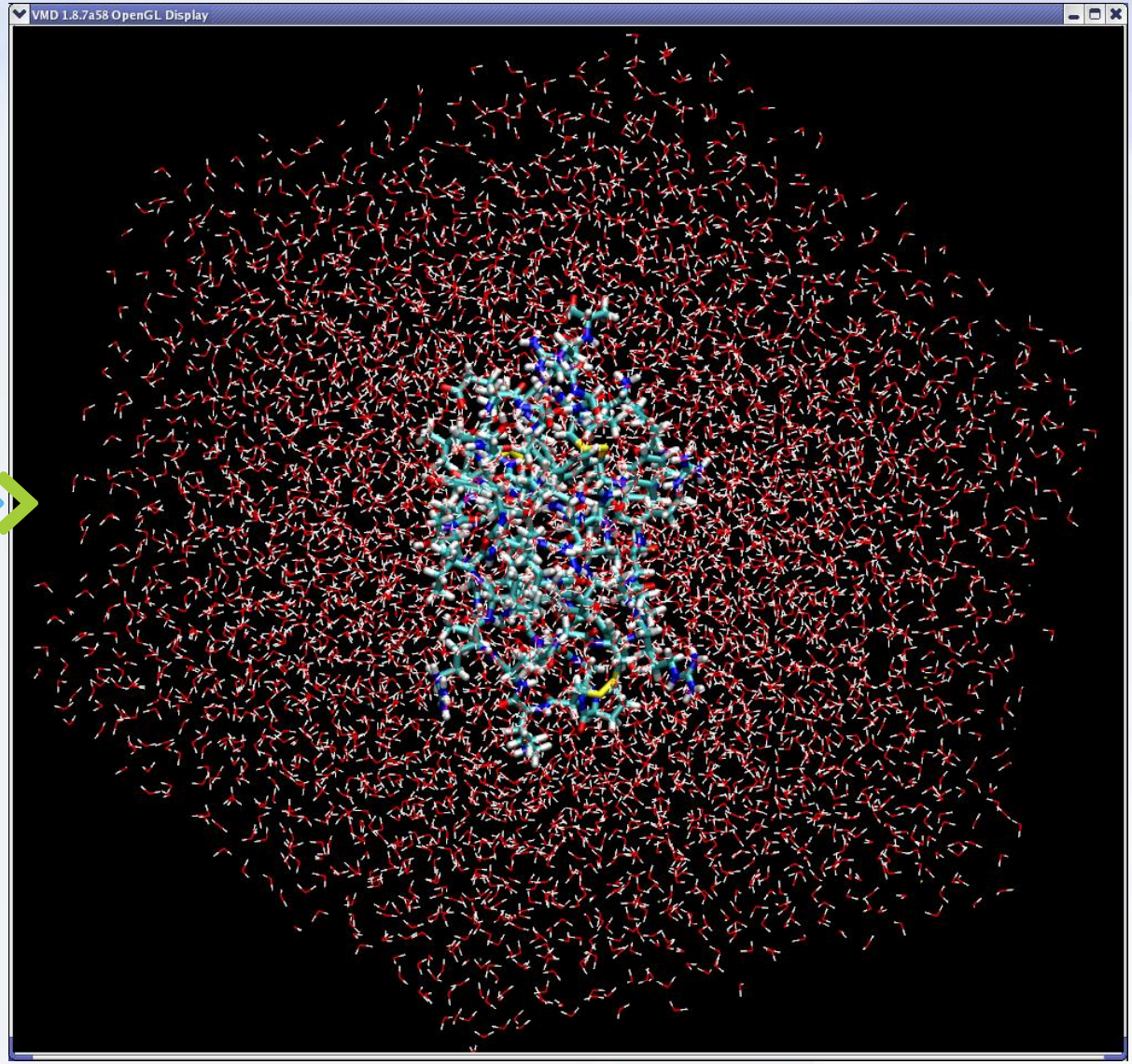
Select the Add solvation box and Add neutralizing ions options



Click the I'm feeling lucky button

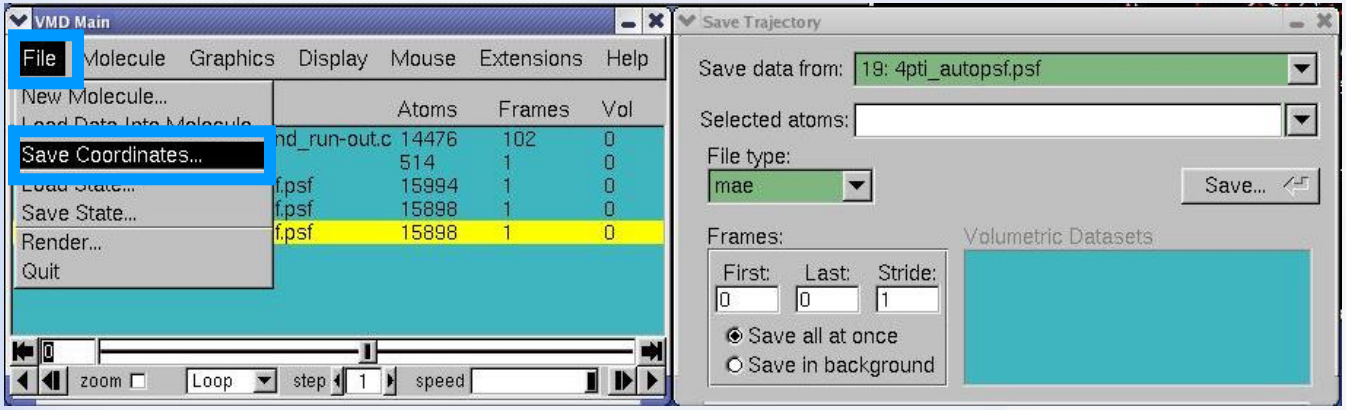
Preparing molecular systems with VMD

The solvated protein appears in the **VMD Display** window



Preparing molecular systems with VMD

Select **File>Save Coordinates**
Select **mae** from the **File Type** field
Give the file a name
Click **Save** >>>>



You can also issue this command from the VMD text window:

```
vmd > animate write mae my_output_file.mae
```

Process the Maestro file with *Viparr* and the **build_constraints** script to add force field parameters and constraints

Loading files into VMD from scripting interfaces

- Start Python:

```
gopython
```

- Load the molecule module and read structure file:

```
import molecule
molid = molecule.read(molid=-1, filetype='mae',
filename='structure.cms')
molecule.read(molid, filetype='dtr',
filename='trajectory.dtr', wait-for=-1)
```

- Specify a range of frames to load:

```
molecule.read(molid, 'dtr', 'trajectory.dtr', beg=5,
end=55, skip=10, waitfor=-1)
```

Organization in VMD

- VMD data is organized into *Molecules* which represent entire molecular systems and associated snapshots
- Molecules are assigned integer keys (`molid`)
- One molecule is tagged as the `top`
- Each molecule has a fixed number of atoms
- Atoms are assigned attributes (e.g., name, type, mass, charge)
- Snapshots are referred to as *frames*
- Each frame holds one set of coordinates for each atom and unit cell dimensions for the frame
- Velocities can be stored if specified by trajectory file

Atom selections in VMD

- Consist of all the atoms in a particular molecule and frame that satisfy a predicate or set of conditions
- Could be different for different frames
- Scripting interface uses atom selection to specify a predicate for an atom selection object
- Atom selection objects created in VMD Python with `atomsel` module

  Import the `atomsel` type

```
from atomsel import atomsel
```

  Create a selection of atoms in the top molecule for the current frame

```
all = atomsel()
```

Atom selections in VMD

```
# Select atoms named ‘CA’ in top molecule in current frame
   ca = atomsel(selection=‘name CA’)

# Select protein atoms in frame 10 of top molecule
   pro10 = atomsel(‘protein’, frame=10)

# Select waters near the protein from molecules 0 and 1
   wat0 = atomsel(‘water and same residue as (within 5 of
   protein)’, molid=0)
   wat1 = atomsel(‘water and same residue as (within 5 of
   protein)’, molid=1)
```

Atom selections in VMD

- The `atomsel` type takes three arguments:
  - `Selection` — Selection predicate
  - `Molid` — Molecule associated with the selection
  - `Frame` — Index of the snapshot referenced by the selection
- Setting the frame attribute

```
n1 = len(wat0)      # number of atoms in the selection
f = wat0.frame      # returns -1
wat0.frame = 20     # makes wat0 reference frame 20
n2 = len(wat0)      # will be equal to n1
wat0.update()       # recompute selection on frame 20
n3 = len(wat0)      # might be different from n1
```

- Changing the frame does not cause the selection to be recomputed
- Only the `update()` method changes the atoms referenced
- Changing the frame does change the result of calculations that depend on the coordinates (e.g. `center()` or `rmsd()`)

Atom selections in VMD

- Extracting data about a molecule for the selected atoms:

```
# Get the number of alpha carbons
num_ca = len(ca)

# Get the residue name of each alpha carbon
resnames = ca.get('resname')

# Set the 'type' attribute of all atoms to 'X'
all.set('type', 'X')

# Compute the center of mass of atoms in frame 10
mass = pro10.get('mass')
com = pro10.center(mass)

# Align pro10 to atoms in frame 0, compute RMSD
pro0 = atomsel('protein', frame=0)
m = pro10.fit(pro0, weight=mass)
pro10.move(m)
rms = pro10.fit(pro0)
```

Atom selections in VMD

- Extracting positions and velocities from frames (expensive):

```
# Get the x, y, z coordinates for atoms in wat1
x = wat1.get('x')
y = wat1.get('y')
z = wat1.get('z')

# Get the z component of the velocity
vz = wat1.get('vz')
```

- Extracting positions and velocities with **vmdnumpy**

```
# Return reference to coordinates
vmdnumpy.positions(molid=-1, frame=-1)

# Return reference to velocities
vmdnumpy.velocities(molid=-1, frame=-1)
```

Atom selections in VMD

- Extracting a subset of coordinates for a selection

```
# Return all positions in top molecule's current frame
pos = vmdnumpy.positions()

# Return index of all CA atoms
inds = ca.get('index')

# Return M x 3 array, where M = len(ca)
ca_pos = pos[inds]
```

Functions from VMD built-in molecule module

- `new(name)` — `molid` of newly created molecule
- `listall()` — list of available `molid` values
- `numatoms(m)` — number of atoms in molecule `m`
- `numframes(m)` — number of frames (snapshots) in molecule `m`
- `get_top()` — `molid` of top molecule; `-1` if none
- `set_top()` — make molecule `m` the top molecule
- `get_frame()` — get current frame for molecule `m`
- `set_frame(m,frame)` — set current frame for molecule `m` to `frame`
- `get_periodic(m=-1, frame=-1)` — dictionary of periodic cell data
- `set_periodic(m=-1, frame=-1)` — set periodic cell keywords
- `get_physical_time(m=-1, frame=-1)` — time value for timestep
- `read(molid, filetype, filename, …)` — load molecule files
- `write(molid, filetype, filename, …)` — save molecule files

Writing structures and trajectories in VMD

- `molecule.write(molid, 'dtr', 'trajectory.dtr', waitfor=-1)`

- `molecule.write(molid, 'dtr', 'trajectory.dtr', beg=100, end=-1, skip=10, waitfor=-1)`

- ```
  sel = atomsel('name CA')
  sel.frame = 45
  sel.write('mae', 'ca45.mae')
  ```
  ```

Analyzing whole trajectories in VMD

- Script runs through frames in a molecule, aligns frames to the first frame, and computes the aligned RMSD for each frame and the averaged position of the alpha carbons

```
from atomsel import atomsel
import molecule, vmdnumpy
import numpy

avg = None                  # holds averaged coordinates
all = atomsel()             # all atoms in current frame
ref = atomsel('name CA', frame = 0)  # reference frame 0
sel = atomsel('name CA')  # alpha carbons, current frame
inds = sel.get('index')   # indexes of alpha carbons
mass = sel.get('mass')    # masses of alpha carbons
rms = [0.]                  # holds RMSD, reference frame
```

Analyzing whole trajectories in VMD

```
def processFrame():
   global avg

   # Align current frame with reference frame
   all.move( sel.fit(ref, mass) )

   # Append new RMSD value
   rms.append( sel.rmsd(ref, mass) )

   # Get positions of CA atoms
   pos = vmdnumpy.positions()
   ca_pos = pos[inds]

   # Accumulate positions into average
   if avg is None: avg = ca_pos[:]  # Make a copy of ca_pos
   else: avg += ca_pos     # Add this frame's contribution

# Loop over frames
n = molecule.numframes(molecule.get_top()
for i in range(1,n ):
   molecule.set_frame(molecule.get_top(), i)
   processFrame()

# Scale the average
avg /= n
```

Documentation Resources

- *Desmond Tutorial*
- *Desmond User's Guide*
- Desmond user community website: http://groups.google.com/group/desmond-md-users/
- Schrödinger's Desmond documentation:
  - *Quick Start Guide:* $SCHRODINGER/docs/desmond/quick_start/des22_quick_start.pdf
  - *Desmond User Manual:* $SCHRODINGER/docs/desmond/user_manual/des22_user_manual.pdf
- Maestro documentation:
  - *Maestro Overview:* $SCHRODINGER/docs/maestro/overview/mae90_overview.pdf
  - *Maestro Command Reference:* $SCHRODINGER/docs/maestro/ref_manual/mae90_command_reference.pdf
  - *Maestro Tutorial:* $SCHRODINGER/docs/maestro/tutorial/mae90_tutorial.pdf
  - *Maestro User Manual:* $SCHRODINGER/docs/maestro/user_manual/mae90_user_manual.pdf
- ASL documentation: $SCHRODINGER/docs/maestro/help/mae90_help/misc/asl.html
- Prime documentation
  - *Prime Quick Start:* $SCHRODINGER/docs/prime/quick_start/pri21_quick_start.pdf
  - *Prime User Manual:* $SCHRODINGER/docs/prime/user_manual/pri21_user_manual.pdf